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*9,416 volunteer abstracts, 18 symposia abstracts, and 64 teaching of neuroscience abstracts.
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<td><strong>Incoming Chairperson</strong></td>
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<td>Urs S. Rutishauser, Ph.D.</td>
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* *ex-officio* denotes members appointed by NIH.
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506. Homeotic Genes and the Control of Development
M.P. Scott .................................................. No Abstract

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*Chaired by: A.C. Howlett* ................................................ 1279

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J.L. Rapoport ................................................... No Abstract

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U. Bellugi .......................................................... No Abstract

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**Theme B: Cell Biology**

| 354. How Cells Keep Time: The Molecular and Cellular Basis of Circadian Rhythms | SYMP. | Mon PM | Wed PM |
| 100. Blood-brain barrier                                                      | Poster | Mon AM  |       |
| 28. Cytoskeleton, transport, membrane targeting I                            | Poster | Mon AM  |       |
| 158. Cytoskeleton, transport, membrane targeting II                           | Poster | Tue AM  |       |
| 457. Gene structure                                                          | Poster |       |       |
| 211. Gene structure and function I                                           | Slide  | Tue PM  |       |
| 360. Gene structure and function II                                          | Slide  | Wed PM  |       |
| 513. Gene structure and function III                                         | Slide  | Thu PM  |       |
| 230. Membrane composition: cell surface macromolecules I                     | Poster | Tue PM  |       |
| 379. Membrane composition: cell surface macromolecules II                    | Poster | Wed PM  |       |
| 27. Neuroglia and myelin I                                                   | Poster | Mon AM  |       |
| 157. Neuroglia and myelin II                                                 | Poster | Tue AM  |       |
| 221. Neuroglia and myelin III                                                | Slide  | Tue PM  |       |
| 455. Neuroglia and myelin IV                                                 | Poster | Thu AM  |       |
| 456. Neuroglia and myelin V                                                  | Poster | Thu AM  |       |
| 101. Regulation of gene expression I                                         | Poster | Mon PM  |       |
| 159. Regulation of gene expression II                                        | Poster | Tue AM  |       |
| 604. Regulation of gene expression III                                       | Poster |       | Fri AM|
| 156. Staining, tract tracing and imaging I                                   | Poster | Tue AM  |       |
| 590. Staining, tract tracing and imaging II                                  | Slide  | Fri AM  |       |
| 603. Staining, tract tracing and imaging III                                 | Poster | Fri AM  |       |

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

<p>| 205. Molecular Mechanisms of Neurotransmitter Secretion | SYMP. | Tue PM |
| 71. Regulation of Ion Channels                             | SYMP. | Mon PM | Thu AM |
| 462. Calcium channels: conotoxins and other ligands       | Poster | Wed AM | Thu AM |
| 309. Calcium channels: molecular biology                  | Poster |       |       |
| 461. Calcium channels: multiple types                     | Poster |       |       |
| 310. Calcium channels: phosphorylation                    | Poster |       |       |
| 29. Calcium channels: physiology and pharmacology I       | Poster | Mon AM |       |
| 142. Calcium channels: physiology and pharmacology II     | Slide  | Tue AM |       |
| 358. Calcium channels: physiology and pharmacology III    | Slide  | Wed PM | Fri AM|
| 606. Calcium channels: physiology and pharmacology IV     | Poster |       | Fri AM|
| 535. Ion channels: cell function                           | Poster |       |       |
| 607. Ion channels: chloride and other                     | Poster |       |       |
| 534. Ion channels: ligand-gated                            | Poster |       |       |
| 31. Ion channels: modulation and regulation I              | Poster | Mon AM |       |
| 383. Ion channels: modulation and regulation II            | Poster | Wed PM |       |
| 435. Ion channels: modulation and regulation III           | Slide  | Thu AM |       |
| 608. Ion channels: modulation and regulation IV            | Poster |       | Fri AM|
| 161. Long-term potentiation: models and mechanisms        | Poster | Tue AM |       |
| 4. Long-term potentiation: physiology and pharmacology I  | Slide  | Mon AM |       |
| 380. Long-term potentiation: physiology and pharmacology II | Poster | Wed PM |       |
| 533. Long-term potentiation: physiology and pharmacology III | Poster | Thu PM |       |
| 160. Long-term potentiation: protein kinases and second messengers | Poster | Tue AM |       |
| 102. Pharmacology of synaptic transmission: hippocampus | Poster | Mon PM |       |
| 103. Pharmacology of synaptic transmission: neurotransmitters | Poster | Mon PM |       |
| 605. Postsynaptic mechanisms                                | Poster |       | Fri AM|
| 444. Postsynaptic mechanisms in neurotransmission         | Slide  | Thu AM |       |</p>
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**Theme D: Neurotransmitters, Modulators, and Receptors**

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**Theme I: Neural Basis of Behavior**

<p>| 278            | Dynamical Behavior of Neural Systems                                         | SYMP. | Wed AM|       |       |       |       |
| 279            | The Neuron Doctrine 1891-1991                                                 | SYMP. | Wed AM|       |       |       |       |
| 269            | Antipsychotics I                                                             | Poster| Tue PM|       |       |       |       |
| 270            | Antipsychotics II                                                            | Poster| Tue PM|       |       |       |       |
| 15             | Biological rhythms and sleep I                                               | Slide | Mon AM|       |       |       |       |
| 262            | Biological rhythms and sleep II                                              | Poster|       |       | Tue PM|       |       |
| 263            | Biological rhythms and sleep III                                             | Poster|       |       | Tue PM|       |       |
| 264            | Biological rhythms and sleep IV                                              | Poster|       |       | Tue PM|       |       |
| 265            | Biological rhythms and sleep V                                               | Poster|       |       | Tue PM|       |       |
| 292            | Biological rhythms and sleep VI                                              | Slide |       |       | Wed AM|       |       |
| 345            | Biological rhythms and sleep VII                                             | Poster|       |       | Wed AM|       |       |
| 346            | Biological rhythms and sleep VIII                                            | Poster|       |       | Wed AM|       |       |
| 490            | Biological rhythms and sleep IX                                              | Poster|       |       | Thu AM|       |       |
| 491            | Biological rhythms and sleep X                                               | Poster|       |       | Thu AM|       |       |
| 565            | Drugs of abuse—alcohol                                                      | Poster|       |       | Th| PM  |       |       |
| 151            | Drugs of abuse—alcohol, barbiturates and benzodiazepines                      | Slide |       |       | Tue AM|       |       |
| 567            | Drugs of abuse—amphetamine                                                  | Poster|       |       | Thu PM|       |       |
| 494            | Drugs of abuse—amphetamine and nicotine                                     | Poster|       |       | Th| PM  |       |       |
| 60             | Drugs of abuse—benzodiazepines                                               | Poster| Mon AM|       |       |       | Fri AM|
| 636            | Drugs of abuse—cellular effects of ethanol                                   | Poster|       |       |       |       | Fri AM|
| 349            | Drugs of abuse—cocaine                                                      | Poster|       |       | Wed AM|       |       |
| 348            | Drugs of abuse—cocaine: antagonists and serotonin                            | Poster|       |       | Wed AM|       |       |
| 61             | Drugs of abuse—cocaine: binding and neurophysiology                          | Poster| Mon AM|       |       |       |       |
| 62             | Drugs of abuse—cocaine: development                                          | Poster| Mon AM|       |       |       |       |
| 267            | Drugs of abuse—cocaine: dopamine                                             | Poster|       |       | Tue PM|       |       |
| 150            | Drugs of abuse—cocaine: genes, molecules and buprenorphine                   | Slide |       |       | Tue AM|       |       |
| 268            | Drugs of abuse—cocaine: monoamines and brain stimulation                     | Poster|       |       | Tue PM|       |       |
| 566            | Drugs of abuse—cocaine: pharmacology                                         | Poster|       |       | Thu PM|       |       |
| 80             | Drugs of abuse—cocaine: transporters and toxins                              | Slide | Mon PM|       |       |       |       |
| 564            | Drugs of abuse—ethanol                                                       | Poster|       |       | Thu PM|       |       |
| 568            | Drugs of abuse—opioids                                                       | Poster| Mon PM|       |       |       |       |
| 132            | Drugs of abuse—opioids: dopamine and dependence                              | Poster| Mon PM|       |       |       |       |
| 635            | Drugs of abuse—prenatal ethanol                                               | Poster|       |       | Fri AM|       |       |
| 197            | Hormonal control of behavior I                                              | Poster|       |       | Tue AM| Wed PM|       |
| 419            | Hormonal control of behavior II                                             | Poster|       |       | Thu PM|       |       |
| 561            | Hormonal control of behavior III                                            | Poster|       |       | Thu PM|       |       |
| 562            | Hormonal control of behavior IV                                             | Poster|       |       | Thu PM|       |       |</p>
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**Theme J: Disorders of the Nervous System**

<p>| 582.          | Neural Grafting and Parkinson's Disease                                      | SYMP.    | Tue PM |      |      |      | Fri AM|
| 206.          | Therapeutic Potential of Neurotrophic Factors                                | SYMP.    | Tue PM |      |      |      |       |
| 576.          | Affective illness and related disorders                                      | Poster   |      |      |      | Thu PM|       |
| 364.          | Alzheimer's disease: amyloid I                                              | Slide    | Wed PM |      |      |      |       |
| 439.          | Alzheimer's disease: amyloid II                                             | Slide    |       |      |      | Thu AM|       |
| 517.          | Alzheimer's disease: amyloid III                                            | Slide    |       |      |      | Thu PM|       |
| 572.          | Alzheimer's disease: amyloid IV                                             | Poster   |      |      |      | Thu PM|       |
| 573.          | Alzheimer's disease: amyloid V                                              | Poster   |      |      |      | Thu PM|       |
| 422.          | Alzheimer's disease: cytoskeleton                                            | Poster   |      |      |      | Wed PM|       |
| 421.          | Alzheimer's disease: experimental models                                     | Poster   |      |      |      | Wed PM|       |
| 83.           | Alzheimer's disease: genetics and growth factors                            | Slide    | Mon PM |      |      |      |       |
| 290.          | Alzheimer's disease: neurochemistry I                                      | Slide    | Wed PM |      |      |      |       |
| 423.          | Alzheimer's disease: neurochemistry II                                      | Poster   |      |      |      | Wed PM|       |
| 498.          | Alzheimer's disease: neuroimaging and diagnostic tests                      | Poster   |      |      |      | Thu AM|       |
| 147.          | Alzheimer's disease: neuropathology I                                       | Slide    | Tue AM |      |      |      |       |
| 271.          | Alzheimer's disease: neuropathology II                                      | Poster   | Tue PM |      |      |      |       |
| 272.          | Alzheimer's disease: neuropathology III                                     | Poster   | Tue PM |      |      |      |       |
| 273.          | Alzheimer's disease: neuropsychology                                        | Poster   | Tue PM |      |      |      |       |
| 274.          | Alzheimer's disease: pharmacology and drug trials                           | Poster   | Tue PM |      |      |      |       |
| 580.          | Clinical CNS neurophysiology                                                 | Poster   |      |      |      | Thu PM|       |
| 574.          | Degenerative disease                                                         | Poster   |      |      |      | Thu PM|       |
| 351.          | Developmental disorders of the nervous system I                             | Poster   |      |      |      | Wed AM|       |
| 495.          | Developmental disorders of the nervous system II                            | Poster   |      |      |      | Thu AM|       |
| 63.           | Developmental genetic models                                                 | Poster   | Mon AM|      |      |      |       |
| 199.          | Developmental genetic models: transmitter systems and second messengers      | Poster   | Tue AM |      |      |      |       |
| 68.           | Epilepsy: animal models I                                                    | Poster   | Mon AM|      |      |      |       |
| 200.          | Epilepsy: animal models II                                                   | Poster   | Mon AM|      |      |      |       |
| 497.          | Epilepsy: anticonvulsant drugs                                               | Poster   |      |      |      | Thu AM|       |
| 67.           | Epilepsy: basic mechanisms I                                                 | Poster   | Mon AM|      |      |      |       |
| 201.          | Epilepsy: basic mechanisms II                                                | Poster   | Mon AM|      |      |      |       |
| 571.          | Epilepsy: basic mechanisms III                                               | Poster   |      |      |      | Thu PM|       |
| 596.          | Epilepsy: basic mechanisms IV                                                | Slide    |       |      |      | Fri AM|       |
| 638.          | Epilepsy: basic mechanisms V                                                 | Poster   |       |      |      | Fri AM|       |
| 396.          | Epilepsy: human studies and animal models I                                 | Slide    | Wed PM |      |      |      |       |
| 496.          | Epilepsy: human studies and animal models II                                | Poster   |      |      |      | Thu AM|       |
| 570.          | Experimental genetic models                                                  | Poster   |      |      |      | Thu PM|       |
| 350.          | Genetic models of behavior                                                   | Poster   |      |      |      | Wed AM|       |
| 503.          | Infectious diseases                                                          | Poster   |      |      |      | Thu AM|       |
| 77.           | Ischemia I                                                                   | Slide    | Mon PM|      |      |      |       |
| 425.          | Ischemia II                                                                  | Poster   | Wed PM|      |      |      |       |
| 426.          | Ischemia III                                                                 | Poster   | Wed PM|      |      |      |       |</p>
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395.7 DECREASED SERTOLIEN (5-HT) SYNTHESIS METABOLISM AND 5-HT1A RECEPTOR DENSITY IN VARIOUS BRAIN REGIONS OF INFLAMMATORY DISEASE SUSCEPTIBLE LEWIS RATS. PHILIP M. BURMAN*, IVAN MEHLEN*, CRAIG C. SMITH*, GEORGE P. CHRUSCH*, PHILIP W. GOLD* AND ESTHER M. STRENBerg. Department of Pediatrics, University of California, San Diego, CA 92103.

395.9 STIMULATION OF HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) ACTIVITY BY HYPOTHALAMIC DA NEURONS. B. BORZI and C. M. RAM, Dept. of Pharmacology, Duke University Medical Center, Durham, N.C. 27710.


396.4 ARGinine vasopressin (AVP) and oxytocin (OT) have been demonstrated to stimulate the pituitary-adrenal axis in a synergistic fashion with CRH, a potent stimulator of the HPA axis. We propose that the therapeutic efficacy of activating drugs in atypical depression may reflect a capacity to increase the expression of the AVP receptor in the LC, an effect that could be theoretically utilized in the screening of pharmacologic agents for the treatment of this disorder.

Corticosteroid factor (CRF) stimulates release of proopiomelanocortin (POMC) peptides, beta-endorphin (END) or alpha-melanocyte stimulating hormone (MSH) from rat neurointermediate lobe (NILS) in vitro. CRF induced peptide release is significantly reduced by dopamine (Saladin et al., '88, Neuropeptides 12: 59) or by the CRF-antagonist, alpha-helical CRF (Saladin et al., '91, Neuropeptides, in press). The benzodiazepine, adinazolam (ADIN) may modulate hormones which affect anxiety by suppressing CRF effects. Here, we examined the ability of ADIN to modulate CRF-induced END release from incubated rat NILS. Adult male Sprague-Dawley rats were ether anesthetized, rapidly decapitated, and the NILS (giganted in vitro for 90 minutes with CRF (10^-6 M), ADIN (10^-5 to 10^-9 M), alone, or CRF followed by ADIN. Aliquots of media were taken for radioimmunoassay of END at 15 minute intervals. At 90 minutes, NILS were fixed and processed for light or electron microscopy (EM). Adinazolam added at 30 minutes suppressed the CRF-induced release of END, while ADIN alone showed non-specific modulation of END release. EM showed well-preserved tissue at the 90 minutes. Adinazolam may act at the level of the pituitary to suppress the stress hormone release induced by CRF. Supported by The Upjohn Co., NIH NS 21156, GM 08139(LCS) and NS 08477 (JAC).

INTERACTIONS BETWEEN CANNABINOIDS AND STEROIDS IN RAT HIPPOCAMPUS. J.C. Elskridge, H. Y.H. Lau, P.J. Enstrom and P.W. Landfield. Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083, and Department of Pharmacology, University of Kentucky School of Medicine, Lexington, KY 40536.

In previous papers, we have examined hormonal and electrophysiological effects of cannabinoids (THC) and corticosterone (CORT) on hippocampal slices. THC at high doses (0.1-1.0 μM) produced a transient (30-60 s) increase in neuronal activity. THC at concentrations of 0.01-0.1 μM produced a late, slow increase in neuronal activity. Adrenalectomy increased THC effects. CORT at 1 nM to 1 μM did not produce significant changes in neuronal activity. In the present study, we assessed the effects of THC and CORT on neurons in hippocampal slices. THC at 0.1-1.0 μM, CORT at 1 nM to 1 μM, and their combination were assessed in hippocampal slices. THC produced a transient increase in neuronal activity which was followed by a slow increase in neuronal activity. CORT produced a slow increase in neuronal activity. The combination of THC and CORT produced a transient increase in neuronal activity which was followed by a slow increase in neuronal activity. These findings suggest that basal BNZ receptor differences between these strains may be secondary to strain differences in corticosterone levels. Since BNZs attenuate hypothalamic CRH secretion through GABAergic action, we suggest that strain differences in receptor number could therefore also augment strain differences in HPA axis function through differential sensitivity to GABA mediated feedback.
HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION I

WEDNESDAY PM

We are investigating factors leading to the induction of the protein product of the proto-oncogene c-fos in the adenocorticotropic hormone (ACTH) secreting corticotrophs. The almost universal elevation of ACTH in vivo and endocrine cells suggests a possible role of Fos in either short or long term regulation of secretion. The gene for Fos contains response elements that are induced by Ca**+, Protein Kinase C (PKC), and MAP kinase. We are investigating the effect of known second messengers which act through these second messengers on ACTH secretion.

In order to more effectively examine Fos in the corticotrophs, a 4-fold enrichment of this cell type has been obtained using cell sorting techniques. Localization of Fos in this enriched culture was performed by immunocytochemistry using a polyclonal antibody corresponding to a Ca**+-2.70 (CRB). Western blots of anterior pituitary homogenates confirm the specificity of the antibody for Fos and its ability to be blocked by the peptide to which the antibody was raised. In all cases, Fos-like immunoreactivity was seen in both the cytosol and nucleus. There is a significant increase in Fos immunofluorescence following the depolarization of these cells with high external K+. This stimulation leads to a rise in intracellular Ca**+ as detected with fura-5AM. Fos staining also increased in response to the PKC activator TPA(100μM). Corticotropin-releasing factor (CRF,100μM), which is known to elevate cAMP, increased Fos immunoreactivity in a number of cells equaling the calculated percentage of corticotrophs in the culture. We are currently working on getting a pure fraction of corticotropes by flow cytometry which will be used to further investigate the factors necessary for Fos induction and its possible role in ACTH secretion.

Supported by a Klingenstein Fellowship to SAD

CARDIOVASCULAR REGULATION: BRAINSTEM MECHANISMS II

396.1
MODELING REVEALS MECHANISMS OF CENTRAL COMPUTATION IN THE BARORECEPTOR VAGAL REFLEX. E.B. Graven*, J.S. Schwebel, J.F. Paton, K.M. Smyth*, and W.T. Rogers, Neural Computation Group, DuPont Co.,Wilmington, DE 19880-0352; and Royal Free School of Medicine, London NW3 2PF.

Computational modeling provides a framework for understanding function in neural systems. We develop models of key cardiovascular reflexes. This hypothesis performs nonlinear dynamical multivariate control, and thus is ideal for comprehending underlying mechanisms for use in artificial systems. Previously (Rogers et al., Soc. Neurosci. Abst. 16:219, 1990) we reported a model of primary baroreceptor afferents which showed how pressure information is encoded and transmitted to the brain. The present work extends this to illustrate how central neurons transform this code by virtue of their intrinsic membrane properties. Model 1st-order baroreceptor neurons are constructed from modified channel kinetics which account for membrane potential trajectories observed in vivo; including delayed response, spike rate adaptation, rebound depolarization and state-dependent response. Baroreceptor afferents are connected to these central neurons with excitatory ligand-gated synapses. Blood pressure recordings driveafferent activity, which in turn modulates central neuronal activity. Resulting patterns are studied in relation to their impact in order to determine the transformation that is computed centrally. Regarding the 1st-order/2nd-order processing, consequences of different patterns of afferent convergence are being explored in simulation. Our preliminary results indicate the transformation of strongly correlated burst activity on the afferents into low-rate activity of 2nd-order cells due to their intrinsic membrane properties. We are now exploring the significance of population responses for parallel processing.

396.2
RHOSTRO-CAUDAL TOPOGRAPHY OF CARDIAC VAGAL INNERVATION IN THE RAT. L.A. Excoffre*, J.S. Schwebel, J.F. Paton and P.P. Wygit, Neural Computation Group, DuPont Co.,Wilmington, DE 19880-0352; and Royal Free School of Medicine, London NW3 2PF.

Chorea-Toxin/Horseradish Peroxidase was injected into four distinct cardiac sites: 1) between the superior vena cava and the aorta; 2) between the aorta and pulmonary artery; 3) at the junction of the aorta and the heart, and; 4) at the crossing of the jugular vein, left ventricle and pulmonary artery. Only the first and fourth sites displayed consistent and abundant retrograde and anterograde transport. Analysis in transverse, sagittal, and horizontal planes revealed that labeled cardiac preganglionic neurons were primarily observed from 1.0 mm to 2.0 mm from the obex. Cells were present bilaterally in three distinct fields: 1) scattered cells in the dorsal motor nucleus of the vagus (nuclear DMV) from 1.0 mm to 2.0 mm from the obex; 2) a dense, rostral group of up to 126 multipolar,stellate cells in the rostral ventrolateral medulla (nuclear DMV) from 1.0 mm to 2.0 mm from the obex; and 3) a caudal group of up to 123 neurons between 0.12 mm and 1.6 mm distributed in the field between the DMV and the NA. These caudal neurons are often closely packed and have large, tortuously shaped, often intertwined dendrites. While the first injection site preferentially labeled the caudal group, the fourth site exhibited a bias for the rostral cells. Cardiac afferents are distributed in a continuum column within the NTS from obes to -3.0 mm. Cardiac, afferent distribution within the mediobasal commissural NTS, and ventrocaudally half way to the central canal. More rostrally, the terminal field migrates to include only the dorsal tier of the commissural NTS. Then, at levels of the obex, where labeling is most dense, it spreads laterally and away from the commissural to the lateral portions of the NTS. This distribution appears to largely overlap that of other cardio-vascular inputs, specifically that of the aortic nerves. The rostro-caudal range of aortic afferents is less extensive and is shifted somewhat rostrally, extending from +0.2 mm to -1.5 mm.

396.3

We previously reported neuronal rhythmic activity (~3Hz) in rat brainstem slices localized to the cardiovascular NTS (Paton et al., Soc. Neurosci. Abst. 16:219, 1990). This region contains a significant part of the A2 amineergic cell group. In the present study we further characterized these neurons by 1) assessing the importance of glutamatergic synaptic drives for their activity in vitro, and 2), using the presence of tyrosine hydroxylase (TH), Application of glutamatergic blockers (APV, 0.5mM or kynurenic acid (1.0mM) produced two distinct types of response. In half of the cells ongoing discharge was unaffected, and these are referred to as auto-active cells (AA). In the other cells rhythmic activity was abolished and these are characterized as sympathetically driven neurons (SD). In the remaining group of cells ongoing discharge was unaffected, and these are referred to as auto-active cells (AA). The other requiring glutaminergic drives for its activity.
396.5 CARDIOVASCULAR SYMPATHOINHIBITORY CELLS FORM AN EXTENDED LONGITUDINAL COLUMN IN CAT MEDULLA. C.W. Dempsey, S.E. Richardson, and C.J. Fontana*. Lab. of Neurosurgery, Tulane Univ. School of Medicine, New Orleans, LA 70112.

Sympathetic depressor cells have been shown in rat and rabbit to be located in the caudal ventrolateral medulla, just posterior to the plane of the obex. A similar locus, in the area has been assumed to exist in cat, based on cardiovascular depression observed during stimulation of the caudal ventral surface of the medulla (Guerzoni, Leurdes, and Lopes, J. Physiol. 345, 1984). However, the sympathoinhibitory area we have reported for cat (Neurosci. Abstr. 14: 92; 1988) lies quite anterior to the plane of the obex, in a location dorsal to the rostral ventromedial medulla. We now report that a survey using glutamate stimulation of the medullary region joining these rostral-dorsal extremes reveals a column of cardiovascular sympathoinhibitory cells extending from -1 to +3 mm with respect to the plane of the obex, at a distance of 3 mm from the midline (i.e., the cat brain stem atlas of Herman). The greater density of these cells lies in the anterior half of this column. Chemical inhibition of this column yields hypertension, tachycardia, and partial loss of baroreflex, with muscimol providing greater blocking effectiveness than kynurenic acid.

396.7 RESPONSES OF LATERAL SEGMENTAL FIELD NEURONS IN THE CAT MEDULLA TO STATIC MUSCULAR CONTRACTION. G.A. Iwamoto and T.G. Waldrop. Deps. of Physiology & Biophysics and Laboratory Biosciences, Univ. of Illinois, Urbana, IL 61801.

Prior studies have shown that neurons in the ventrolateral medulla are involved in mediating the cardiovascular responses to static muscular contraction; this area is known to have connections with sympathoexcitatory neurons in the lateral tegmental field (LTF). The purpose of the present study was to determine if static muscular contraction alters the discharge frequency of neurons in the LTF. Single unit responses of LTF neurons to static contraction of hindlimb muscles (evoked by hindlimb extension and flexion) were evaluated in anesthetized cats. Computer averaging analyses were used to examine the basal discharge of the studied neurons relative to cardiovascular (cardiac cycle and/or respiratory nerve discharge) or sympathetic (phasic nerve discharge) activity. Muscular contraction elicited increases in the discharge rate of 59% of the LTF neurons studied; 74% of these neurons had a discharge related to phasic nerve activity. Only four percent of the LTF neurons had a discharge related to phasic nerve activity; only ten percent of the neurons with an inspiratory-related discharge were stimulated by muscular contraction. Baroreceptor stimulation (produced by a phenylephrine-induced increase in arterial pressure) elicited a decrease in the firing rate in 58% of the neurons. These results indicate that cardiovascular neurons in the LTF are excited by static contraction of hindlimbs muscles. Thus, the lateral tegmental fields may be part of the supraspinal circuitry involved in regulating cardiovascular responses during exercise. (Supported by NIH 06296; American Heart Association).


Lesion of rostral ventrolateral medulla (RVLM) produces a marked hypertension in conscious SHR. This study examined the effects of these lesions on baroreceptor reflex function in conscious SHR. Lesions were produced via bilateral microinjections of 30 nmol/200 nl of N-methyl-D-aspartic acid (NDMA). Baroreceptor reflex function was assessed in conscious rats using reflex-mediated changes in heart rate elicited by graded i.v. doses of phenylephrine and sodium nitroprusside. reflex function was analyzed using exponential curve fitting analysis. One day post-lesion, the RVLM-lesioned rats showed a significant hypertension in comparison to sham rats (-173±8 vs -176±7 mmHg). The baroreceptor gain in the lesioned rats was significantly reduced when compared to sham rats (-2.13±0.3 vs -4.15±0.5 bpm/mmg). 1 day post-lesion, but completely recovered 6 days post-lesion. These data suggest that RVLM neurons play an important role in baroreceptor reflex function. Recovery of baroreceptor reflex function may reflect increased activity in other spinal or supraspinal networks. (Supported by HL-14386 & 64456)


We previously reported that chronic N-methyl-D-aspartic acid (NDMA)-induced lesion of neurons in RVLM causes profound hypertension in conscious SHR. The aim of the present study was to examine the effects of these lesions on cardiovascular reflex function in conscious SHR. Lesions of RVLM were produced by bilateral microinjections of 300 nl (200 nl/rat) of 10 mM NDMA. Cardiovascular reflex function was tested by intravenous (iv) injection of 5-hydroxytryptamine (5-HT). One day post lesion, low doses of 5-HT (200 ng/kg) elicited increases in heart rate (249±20 bpm) in sham and baroreceptor lesioned (375±22 bpm) in lesion rats. The Bezold-Jarisch reflex (hypotension and bradycardia) elicited by intravenous (iv) injection of 100 ng/kg 5-HT was significantly enhanced in lesion rats as compared to sham rats (heart rate: -53±26 vs -24±26; mean arterial pressure: -24±18 vs -11±18 mmHg). The significant differences in reflex function were observed between lesion and sham rats 6 to 15 days post lesion, even though arterial pressure in the lesion rats was higher than in sham rats 6 to 15 days post lesion. These data indicate that neurons in RVLM 1) are responsible for the reflex tachycardia elicited by low doses of 5-HT in control rats and 2) the reflex responses to iv 5-HT. (Support HL-14388 & 64456)


The pathway mediating the prominent presor response elicited by electrical or chemical stimulation of the parabronchial nucleus (PB) is unknown. We injected the calcium channel blocker cobalb chloride (COB) which reversibly blocks sympathetic nerve function onto sites in the ventrolateral medulla to determine their role in mediating this response. Arterial blood pressure (AP), heart rate and sympathetic (renal) nerve discharge (SNR) were continuously monitored. Cardiac output (CO) was calculated using the Fick equation. Presor sites in the PB were either electrical or chemically (D,L-homocysteic acid; DLH) stimulated before and after bilateral injections of 300 nl COB (30 mM) into 10 mm (rat) or the ventrolateral medulla (cat) to confirm the role of COB in mediating this response. 3) Both sites had a similar COB injection protocol. COB injection into the rostral or caudal to the rVLM did not significantly affect the AP and SND responses. Bilateral lesions of the rVLM with the excitotoxic kainic acid (KA; 50 nl, 50 nl) permanently blocked the PB presor response. DLH injections into the effective areas in the rVLM, prior to COB or KA injections, characterized them as potent presor sites.

The presor response elicited by electrical stimulation of the PB was mediated by sympathetic preganglionic neurons in the spiral cord via the pressor region in the rVLM. This suggests that the PB provides an excitatory drive to rVLM presor cells. (Supported by the Heart and Stroke Foundation of Ontario)
GABAB-receptors are involved in this action of EtOH. (Supported by NHLBI grants HL-6835 and HL-38535 and the AHA).

Angiotensin II (Ang II) acts in the dorsal medulla to modulate baroreflex function. Moreover, Ang II binding sites are associated with presynaptic vasoconstrictor fibres in the nucleus tractus solitarii (NTS) and vagal efferent neurons in the dorsal motor nucleus of the vagus (dmX). High resolution "wet" autoradiography now documents the relationship of putative Ang II receptors to the cytoskeletal apparatus of Ang II containing 14 μm diameter medullary sections were processed for film or emulsion autoradiography with 0.5-1 nM 125I-

The effects of EtOH on baroreflex bradycardia and on cardiovascular responses mediated by GABA A and GABA B receptors in the juxtafacial PGi were studied in urethane-anesthetized rats. EtOH, 1-3 g/kg administered i.v. or 25-200 mmol microinjected bilaterally into the juxtafacial PGi reduced the reflex baroreflex elicited by graded i.v. doses of phenylephrine in control animals but not in rats pretreated with 3-mercaptopyrrolidone, a GABA-depleting agent. Mucosal, 10-40 pmol intra-DVC, caused pressor response and inhibited baroreflex bradycardia. The pressor response to mucosal was potentiated and a tachy- cardiac response was observed when muscimol was microinjected into the juxtafacial PGi. Bicuculline, 10 pmol intra-DVC, inhibited the responses to muscimol but not to baclofen, and reduced the baroreflex inhibitory action of EtOH. Hydroxy-saclofen, 400 pmol intra-DVC, inhibited the responses to baclofen but not to muscimol, reduced the baroreflex inhibitory action of EtOH and prevented the EtOH-potentiation of the baroreflex inhibitory action of EtOH. 2-Hydroxy-saclofen, but not to muscimol, reduced the baroreflex inhibitory action of EtOH. 2-Hydroxy-saclofen, 400 pmol intra-DVC, inhibited the responses to muscimol but not to baclofen, and reduced the baroreflex inhibitory action of EtOH. Hydroxy-saclofen, 400 pmol intra-DVC, inhibited the responses to baclofen but not to muscimol, reduced the baroreflex inhibitory action of EtOH and prevented the EtOH-potentiation of the baroreflex inhibitory action of EtOH.

EtOH inhibited baroreflex bradycardia through the potentiation of the actions of the baroreflex from its afferents. Both GABA A and GABA B-receptors are involved in this action of EtOH.
CARDIOVASCULAR REGULATION: BRAINSTEM MECHANISMS II


The ventricular cardioauditory (VLM) and parabulbar (PBN) complex have been shown to be involved in central cardiovascular regulation. This study was done to determine the distribution of fibers and terminals in the PBN complex originating from neurons within VLM in the rat. Fibers and terminals in PBN from the caudal (c) VLM, rostral (r) VLM and intermediate (i) VLM were examined using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). PHA-L was iontophoresed into the cVLM, rVLM, or iVLM, and after a survival period of 7-10 days, transverse sections of the brainstem were processed using the tetramethyl-benzidine method. PHA-L labelled cells within the injection sites were observed to overlap the A1 and C1 catechol-amnergic cell groups in the cVLM, rVLM or iVLM. PHA-L labelling in the PBN complex resulting from a cVLM injection was observed primarily in the caudal two third of K6/7-like fusiform nuclei (K6) and the parabrachial (PBN) complex. These data have provided the first anatomical demonstration of the connection between these structures. Additionally, sparse labelling was observed in the subnuclei of the complex. Furthermore, these data indicate that clonidine’s inhibition of the cardiovascular response to PAG stimulation is primarily mediated by an evoked increase in circulating epinephrine or other humoral factors. These data suggest that within a restricted region of NTS there exists a pool of neurons sensitive to changes in sodium concentration of the extracellular fluid, and when activated by the sodium these neurons elicit cardiovascular responses as a result of sympathetic inhibition. (Supported by MRC and HSFO)

Carotid baroreceptor projections to the nucleus of the solitary tract in the rat. J. Cinel. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The medullary connections of the carotid baroreceptors were studied in the rat using the tracer Phaseolus vulgaris leucoagglutinin (PHA-L) in the nucleus of the solitary tract (NTS). A microinjection of PHA-L into the carotid sinus nerve bilaterally in 33 animals was found to be associated with dense terminal labeling in the rostral part of the NTS. These data suggest that there are two populations of neurons within the central nervous system that are responsible for processing information from the carotid baroreceptors.

Factors mediating the hindlimb bloodflow component of the defense reaction and their sensitivity to clonidine. L.L. Watkins and W. Mainprize. Dept. of Pharmacology and Dental Research Center, University of North Carolina, Chapel Hill, N.C. 27514.

We previously reported that clonidine inhibits the increase in hindlimb bloodflow (HLBF) associated with the cardiovascular defense reaction elicited by picrotoxycutic acid (PA) stimulation in the rat (Neurosci. 1986; 5:154-1999). The present experiments were designed to examine potential mechanisms contributing to this increase in HLBF to maximize its sensitivity to inhibition by clonidine. First, the role of epinephrine in the HLBF response was evaluated by measuring perfusion pressure and changes in HLBF and to examine their sensitivity to inhibition by clonidine. The present experiments were designed to examine potential mechanisms contributing to this increase in HLBF is possibly associated with inhibition of epinephrine release from the adrenal glands. (Supported by DE80123 & RR07333)


The nucleus ambiguous (AMB) is known to contain vagal cardioauditory neurons. In a previous study we have shown that microinjection of neurotensin (NT) into AMB elicits a vagal bradycardia. In this study, the possibility that NT and L-glutamate (Glu) microinjected into AMB (10-25nl) had a synergistic effect on the heart rate (HR) was investigated. In addition, the effect of NT or Glu microinjections into AMB on the reflex vagal bradycardia elicited during activation of baroreceptors (l-phenylephrine, 100ng/ml) was investigated. Experiments were done in alpha-chloralose anesthetized, paralyzed and artificially ventilated male Wistar rats that had their spinal cord cut at the level of C2. Microinjections of Glu (10-25ml) or NT (50-125ml) elicited decreases in HR of 51±7 to 31±7 bpm, respectively. Combined microinjections of Glu and NT elicited a decrease in HR (82±12 bpm) that was not significantly different from the algebraic sum of the individual Glu and NT responses. In addition, microinjection of Glu significantly increased (88±20 bpm), whereas NT did not alter (39±45 bpm), the magnitude of the reflex vagal bradycardia elicited during baroreceptor activation. Taken together, these data suggest that Glu and NT act as neurotransmitters or modulators in the baroreceptor reflex pathway to AMB and that NT may exert a selective effect on vagal cardioauditory neurons, whereas Glu may also activate other neuronal systems contributing to the reflex response. (Supported by MRC of Canada and HSFO)


The nucleus tractus solitarius (NTS) is known to play an important role in the integration of cardiovascular afferent information. In this study, NTS was systematically explored for cardiovascular responsive sites during microinjections (20nl) of phospholipid buffered saline (PBS, pH 7.0) containing 142mM NaCl, 179mM NaHCO3, 2.5mM CaCl2, 1.3mM MgCl2, 5.6mM KCl, 25.3mM glucose, and 1mM HEPES buffer. The effects of increased plasma arterial pressure (AP; range of means, 21±3 to 53±9 mmHg) were examined in 12 animals. Microinjections into the NTS elicited selective cardiovascular responses. Administration of atropine methyl bromide had no significant effect on the magnitude of the AP and HR responses. However, administration of hexamethonium bromide abolished both the AP and HR responses. Control microinjections (20nl) into the caudal medullary subnucleus of NTS increased arterial pressure by 25±5 to 43±7 mmHg. Microinjection of NT containing no NaCl of NTS did not elicit cardiovascular responses. These data suggest that within a restricted region of NTS there exists a pool of neurons sensitive to changes in sodium concentrations of the extracellular fluid, and when activated by the sodium these neurons elicit cardiovascular responses as a result of sympathetic inhibition. (Supported by MRC of Canada and HSFO)


Previous studies indicated that neural reflexes may control ANF secretion (Baertschi et al., AJP, 199, 90). The purpose of the present study was to investigate whether the rostral ventrolateral medulla (RVL), a source of central sympathetic drive, can modulate the plasma level of atrial natriuretic factor (ANF). Anesthetized, artificially ventilated rats, electrical stimulations were stereotaxically applied to RVL. Bilateral electrical stimulation of RVL caused a 94 ± 18% increase in plasma ANF over baseline (P < 0.01, n = 11), whereas sham stimulation of RVL elicited no change in plasma ANF. Bilateral microinjections of L-glutamine into RVL of artificially ventilated rats elicited an 81 ± 12% increase (P < 0.01, n = 11). Electrical stimulations rostral and dorsal to RVL had no effect on plasma ANF. Unilateral microinjections of L-glutamine into RVL of artificially ventilated rats elicited an 81 ± 12% increase (P < 0.01, n = 11). These results suggest that RVL contains a population of neurons which may modulate plasma ANF. These findings may be of clinical importance. (Supported by AHA grant VA-99-F-9 and VA-90-G-68)
397.1 HYPOTHALAMIC TRANSPORT OF ANGIOTENSIN(1-7). C.L. Block and R.H. Villack*. Cleveland Clinic Foundation, Cleveland, OH 44195-5070.

Several studies have described a close anatomical association of angiotensin (Ang)(1-7) with the hypothalamic vasopressin system. Specifically, Ang(1-7) is localized by immunocytochemistry in neurons of the paraventricular (PVN) and supraoptic nuclei. Vascular immunoreactive fibers emanate from the PVN in the paraventriculo-neurohypophyseal tract (PVN) and periventricular region. In this hypothalamo-neurohypophyseal system, anatomical changes suggestive of transport of Ang(1-7) occur in responses to 40 min-dehydration in a similar manner to vasopressin, and Ang(1-7) is not visualized in the homozygous Brattleboro rat. The current study addresses whether Ang(1-7) is transported from the PVN to the neurohypophysis. Stereomicroscopic views were placed through the PVN in pentobarbital-anesthetized rats. The animals were perfused transcardially 24, 48, or 72 hours later and brain tissue was processed for immunohistochemistry of Ang(1-7).

Unilateral knife-cuts placed lateral to the PVN, severing part or all of the PVN, failed to engorge with Ang(1-7) immunoreactivity for 40 min-dehydration in a similar manner to vasopressin. Neither BP nor HR were significantly altered by aCSF or insulin. Basal levels of Ang(1-7) were significantly decreased in the PVN of rats treated with insulin. This result suggests that Ang(1-7) is transported from the PVN to the neurohypophysis as a function of dehydration.


Guanethidine (Gu) produces chemical sympathectomy in rats (GuX). We explored the baroreflex and vasopressin (AVP) responses as potential mechanisms for maintenance of arterial pressure (AP) in GuX rats. Rats (350-450 g) were anesthetized with ketamine and xylazine, and were cannulated for measurement of AP and heart rate (HR), for IV bolus of salt (0.3 M NaCl) for 30s, and for plasma AVP sampling. In conscious rats, 0.05% GuX was infused at a rate which maintained arterial BP for 120 min, and was slightly elevated over control levels.

As compared to controls, GuX showed a similar resting AP and AVP responses (P<0.05) compared to controls. A second study was performed to determine if GuX rats exhibit a decrease in AP and AVP responses to control levels. AP and AVP responses were similar to control levels. These results suggest that the baroreflex and AVP responses are not altered in GuX rats. GuX may contribute to AVP responses to cardiovascular afferrent inputs.

397.3 CENTRALLY ADMINISTERED INSULIN ALTERS PLASMA CATECHOLAMINE LEVELS BUT NOT BLOOD PRESSURE OR HEART RATE. J. Markus, R.S. Odegard, J.H. Surerude and L.A. Pessah. Department of Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7764.

The present studies investigated the effect of insulin delivered centrally on plasma norepinephrine (pNE), and epinephrine (pEPI), blood pressure (BP), and heart rate (HR) in rats. Insulin (10 μU/mg) or artificial cerebrospinal fluid (aCSF) was infused into the third ventricle of Sprague-Dawley rats (n=6) for 60 min. Neither BP nor HR were significantly altered by aCSF or insulin. Basal levels of pNE did not differ between aCSF and insulin groups (201.7 ± 85.3 pg/ml, aCSF: 201.1 ± 81.4 pg/ml). Insulin significantly decreased pEPI (aCSF: 166 ± 24.6; insulin: 217.6 ± 42.8 pg/ml). Insulin significantly decreased plasma insulin levels (397.1 pg/ml).

397.4 Cardiovascular and Behavioral Effects of Fatal Hypothalamic-Infraoptic Lesions in Hypertensive and Normotensive Syrian Hamsters. X. Chen and M. Swanson. University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7764.

Several studies by our group suggest a close anatomical association of vasopressin, and Ang-(1-7) is not visualized in the homozygous Brattleboro rat. The current study addresses whether Ang-(1-7) is transported from the PVN to the neurohypophysis. Stereomicroscopic views were placed through the PVN in pentobarbital-anesthetized rats. The animals were perfused transcardially 24, 48, or 72 hours later and brain tissue was processed for immunohistochemistry of Ang-(1-7).

Unilateral knife-cuts placed lateral to the PVN, severing part or all of the PVN, failed to engorge with Ang-(1-7) immunoreactivity for 40 min-dehydration in a similar manner to vasopressin. These results suggest that Ang-(1-7) is transported from the PVN to the neurohypophysis as a function of dehydration.

397.5 CORTICOSTEROID BINDING IN LIMBIC BRAIN REGIONS OF NORMOTENSIVE AND HYPERTENSIVE SYRIAN HAMSTERS. X. Chen and M. Swanson. University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7764.

While Ang-(1-7) immunoreactivity in the neurohypophysis was less intense from control animals, no anatomical changes in the peptide distribution were observed.

The data from this study supports the hypothesis that Ang-(1-7) is transported in the PVN to the neurohypophysis. However, the possibility that angiotensin precursor is taken up and processed to Ang-(1-7) by the neurohypophysis is not surprising since much of the fiber input arises from the supraoptic nucleus.

[Supported by NIH HL-69357, HL 37927, and HANEO]
397.7 SALT-INDUCED HYPERTENSION IN DAHL RATS IS ASSOCIATED WITH ENHANCED EXPRESSION OF VASOPRESSIN mRNA WHICH IS MODULATED BY ANGIOTENSIN II AND ALPHA-2 ADRENERGIC RECEPTORS. Bang H. Wung, Timothy Peters* and Prince Chan*. Terre Haute Heart Center for Medical Education, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Central mechanisms for salt-induced hypertension are not clear. Dahl salt-resistant and salt-sensitive (SS) rats were fed with high-salt diet or vasopressin (VP) oligonucleotide probe to study VP mRNA after in situ hybridization, and 125I-angiotensin II (AII) and -iodoclonidine ligands to label AII receptors and alpha-2 adrenergic (A2) receptors respectively. VP mRNA, AII and A2 receptors were measured using quantitative autoradiography. Results showed that hypertension was developed in SS rats whose hypothalamic AII receptors and VP mRNAs were significantly increased, whereas A2 receptors were also up-regulated. This study suggests that salt-induced hypertension is associated with enhanced VP mRNA expression which is controlled by AII receptors and modulated by A2 receptors. Supported by PHS grant NS29587.

397.8 NEUROTENSIN-MEDIATED MODULATION OF NEURAL RESPONSES IN THE LATERAL HYPOTHALAMIC AREA (LHA) TO STIMULATION OF CARDIOVASCULAR SITES IN THE INSULAR CORTEX (IC). G.V. Allojo and D.F. Cechetto. Roberts Research Institute, University of Western Ontario, London, Ontario, Canada, NSA SK8.

Sym pathetic responses elicited from the IC via a synaptic relay in the LHA (Cechetto and Chen, '80). This study examines, in the anesthetized rat (chloral hydrate-urethane), the effects of iontophoresis of neurotransmitter (NT) on excitability of hypothalamic neurons responding to electrical stimulation of the IC (500 μA. 0.5-0.7 Hz). Of 82 spontaneously firing neurons, 32 units (39%) responded to electrical stimulation of cardiovascular pressure or depressor sites in the IC. Of these, 9 units (28%) were excited, 11 units (34%) showed excitation followed by inhibition, 9 units (28%) were inhibited and 3 units (9%) showed inhibition followed by excitation. Iontophoresis of 0.1-1.0 mM NT (25-100 nA) potentiated 11 (34%) of the excitatory responses and attenuated 2 (6%) of the inhibitory responses. Three of the neurons that responded to IC stimulation and six of the neurons that did not respond to IC stimulation exhibited an increased firing rate to NT iontophoresis. These findings indicate that NT may play an important role in the neuromodulation of cardiovascular regulatory commands from the insular cortex. Supported by the Heart and Stroke Foundation of Canada.


Department of Physiologia, Dentistry School, UNESP, Araraquara 14000 SP, Brazil.

Intracerebroventricular (ICV) injection of the cholinergic agonist carbachol (CARB) in rats produces natriuresis, kaliuresis, water intake and hypertension. In this study we investigated the effect of lesion of the medial septal area (MSA) in these responses. Male rats with sham or electrolytic lesion of the MSA were implanted with chronic stainless steel cannula into the lateral ventricle. ICV CARB (8 nmol) in sham rats induced pressor response (35 ± 5 mmHg), diuresis (18 ± 1.5 ml/120 min), natriuresis (55 ± 3.6 ml/120 min) and kaliuresis (78 ± 17 μEq/120 min) responses. Acute (1-7 days) MSA lesions reduced the pressor (18 ± 3 mmHg), natriuresis (23 ± 58 μEq/120 min) and diuresis (5 ± 1.3 μEq/120 min) responses. Chronic lesions (14-18 days) reduced only pressor and natriuretic responses. Kaliuresis was not changed by MSA lesions. These results suggest that MSA plays an important role on the pressor and natriuretic responses induced by central cholinergic activation in the rat. (Research supported by FAPESP).


Pressor response to common carotid occlusion (CCO) produced by implanted occlusion cuffs during 60 seconds in conscious rats have two components: an initial peak (IP) and a maintained response (MR), that is, a plateau of lower intensity observed during the last 30 seconds of occlusion, which does not depend on carotid intima erosion and is probably of central origin. The present study was performed to investigate the role of the paraventricular nucleus of the hypothalamus (PVH) to the development of the pressor response to CCO. Acute (6 hours) and chronic (6 days) lesions of PVH decreased IP by 24% (P<0.05) and MR by 28% (P<0.01). Chromic PVH lesions (6 days) produced similar reductions (IP = 122 ± 20, MR = 22 ± 1.5 mmHg). Aortic denervation, performed 6 hours before CCO, increased the pressor responses observed in chronic PVH lesioned rats (IP = 125 ± 5, MR = 62 ± 6, MM = 32 ± 5 mmHg). These data suggest that PVH plays a role in the integration of the pressor response to carotid occlusion and that the important buffer effect of the aortic baroreceptors on the cerebral circulation is still present after PVH lesion. (Research supported by CNPq: 303802/85-5).

397.11 HYPOTHALAMIC GABAERGIC INFLUENCES ON TREADMILL EXERCISE RESPONSES IN RATS. J.M. Overton, R.W. Hedding, S.L. Yancey, and R.W. Stremel. Dept. of Physiology & Biophysics and Exercise Physiology, University of Louisville, Louisville, KY 40292.

To test the hypothesis that a GABAergic mechanism within the posterior hypothalamus (PH) mediates the cardiovascular adjustments to dynamic exercise in conscious animals, Sprague-Dawley rats were chronically instrumented with bilateral guide cannula directed at the PH, a carotid or femoral cannula, and Doppler probes placed on the mesenteric and iliac arteries. Saline (SAL, 100 nl/side) or the GABA agonist, muscimol (MUS, 125 ng/100 nl), was injected into the PH during steady-state treadmill exercise (20 m/min). SAL had no effect on mean arterial pressure (MAP), heart rate, mesenteric resistance (MR) or iliac resistance (IR) during exercise. MUS had no consistent effect on MAP (2 ± 1 %), HR (2 ± 4 %), MR (2 ± 4 %) or IR (2 ± 4 %), but produced a significant increase (22 ± 7 %) in IR. In addition, hypothalamic muscimol reduced exercise run time, but did not produce changes (sedation) most evident post-exercise. The data suggests hypothalamic GABAergic modulation of exercise in PH. Supported by Univ. of Louisville Graduate School.


This study was designed to determine the effect of electrical stimulation of the preoptic region and hypothalamus on adrenolumbar (AL) venous flow and plasma catecholamine (CA) secretion in the cat. Electrode sites (N=75) were chosen in awake, restrained cats (N=16) that elicited signs of sympathoadrenal activation (e.g., hissing, pupillary dilatation). Later, under methoxyflurane anesthesia, cannulae were placed in the left jugular and left femoral veins and arterial. AL venous samples were collected by gravity during the 3 consecutive 1-minute periods: 1) prior to stimulation and 15 minutes after implantation of the stimulating electrode; and 1) prior to stimulation and 15 minutes after stimulation onset (square-wave, biphasic pulses, 60 Hz, 0.6 mA; 30 sec). CA levels were determined by radioenzymatic assay using COMT and S-adenosyl methionine [methyl-14C] secretion was expressed as ng/min. Hypothalamic stimulation most frequently increased the secretion of both NE and EPI, although evidence of preferential secretion was observed. Stimulation of 17 sites, primarily in the medial hypothalamus and nucleus of the diagonal band, increased AL flow > 12% baseline and elicited significantly greater increases in EPI secretion. These data are consistent with the hypothesis that secreted EPI acts locally to increase blood flow through the adrenal medulla.
397.13  
CALCITONIN GENE-RELATED PEPTIDE (CGRP) MODULATION OF VISCERAL AND NOCICEPTIVE INPUTS TO THE VENTRAL BASAL THALAMUS (VBT). R. Z. Liang and D. F. Cechetto. Roberts Research Institute, University of Western Ontario, N6A 3K8.

Visceral and nociceptive afferents terminate in the VBT. CGRP has been demonstrated to be released by nociceptive inputs to neurons in the parabrachial nucleus to the VBT. The effects of intrathecal application of CGRP on the responses of extracellularly recorded thalamic neurons to activation of visceral and nociceptive inputs were investigated in chloralose-anesthetized rats. Few neurons (39/800, 5%) were observed to change their spontaneously firing rate in response to application of CGRP (Group I, n = 3) and saline (Group II, n = 3) into the VBT. The responses of 120 neurons sensitive to baroreceptor activation (phenylephrine i.v.) of a total of 48 tested; 6/15 to gastric mechanosensory activation (gastric balloon inflation) of a total of 33 tested; 5/12 to taste stimuli (NaCl on the tongue) of a total of 31 tested; 11/18 to pain stimuli (cutaneous pinch) of a total of 59 tested. In addition, nociceptive inputs converged on a large proportion of the visceral responsive neurons. These results suggest that CGRP serves as a major modulator of visceral and nociceptive inputs to neurons in the ventral basal thalamus. Supported by the Heart and Stroke Foundation of Ontario.

397.15  
HYPOTHALAMIC EXCITATORY AMINO ACID (EAA) RECEPTORS MEDIATE STRESS-INDUCED TACHYCARDIA. R. P. Bolton and J. A. D'Incarno, Dept. of Pharmacology and Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Microinjection of the GABA-A receptor agonist muscimol into the dorsomedial hypothalamus (DMH) in rats prevents the tachycardia and hypertension seen in an air-stress paradigm. Here, we examined the effect of injecting EAA antagonists into the DMH on heart rate (HR) and blood pressure during air stress. Conscious chronically instrumented rats received bilateral injections (100 nl/side) of either artificial CSF (ACSF, Groups I and II, n = 12 each), the non-selective EAA receptor antagonist kynurenic acid (KYN, Group III, n = 4 each dose), the NMDA receptor antagonist AP5 100 μmol, the non-NMDA EAA receptor antagonist CNQX 50 μmol or both AP5 and CNQX (Group IV, n = 4 each treatment). Below are means ± SEM of basal HR (beats/min) and changes from basal, HR 3 min after start of stress (5 min post injection).

<table>
<thead>
<tr>
<th>Group</th>
<th>Change</th>
<th>Basal Change</th>
<th>HR Increase (%)</th>
<th>AP5</th>
<th>CNQX</th>
<th>AP5 + CNQX</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5%</td>
<td>315 ± 7</td>
<td>19 ± 3</td>
<td>145 ± 9</td>
<td>154 ± 4</td>
<td>14*</td>
</tr>
<tr>
<td>II</td>
<td>3%</td>
<td>315 ± 7</td>
<td>19 ± 3</td>
<td>315 ± 5</td>
<td>104 ± 5</td>
<td>3*</td>
</tr>
<tr>
<td>III</td>
<td>5%</td>
<td>330 ± 7</td>
<td>28 ± 7</td>
<td>327 ± 12</td>
<td>102 ± 7</td>
<td>3*</td>
</tr>
<tr>
<td>IV</td>
<td>5%</td>
<td>330 ± 7</td>
<td>28 ± 7</td>
<td>327 ± 12</td>
<td>102 ± 7</td>
<td>3*</td>
</tr>
</tbody>
</table>

* different from ACSF treatment by ANOVA (P < 0.05)

These results suggest that activity at EAA receptors in the DMH is required for the generation of stress-induced tachycardia (Supported by USPHS grants 5 R01 NS 39771 and NS 19883, and the American Heart Assoc., Ind. Aff.)

397.17  

Daily exposure to 2 hours of shock-shock conflict or 2 hours of air-jet stress (AJS) has been shown to cause sustained elevations of blood pressure in borderline hypertensive rats (BHR). It is of interest to determine the effects of stress sessions would be effective in causing comparable increases in blood pressure in the current study, the effect of 20 min of daily AJS on blood pressure was contrasted with the effects of 120 min of AJS. At eight weeks of age, male BHR and Wistar rats were subjected to either 20 or 120 min of AJS for 5 days/week for five weeks. Air-jet stress consisted of a stream of air directed toward the top of the head. Maturational changes in blood pressure were observed for the first time in response to the stressor of the tachycardia (Supported by the Heart and Stroke Foundation of Ontario).

397.14  
FOREBRAIN 5-HT RECEPTORS CAUSE SYMPATHOEXCITATION IN RATS. A. L. Ramsey, J. K. Anderson* and C. R. Martin*. Deps. Pharmacology, Royal Free Hospital School of Medicine, London, W3 2FF and Wellcome Laboratories, Kent, UK.

Lateral ventricular (i.c.v.) application of 5-HT causes a rise in blood pressure which is thought to be due to activation of 5-HT receptors located in the anterior hypothalamus/preoptic region. The nature of the receptors mediating this pressor effect was investigated. In chloralose-anesthetized rats recordings were made of BP, HR, renal (RNA) and phrenic nerve activity (PNA). 5-HT (40, 120 nmol kg^-1), DP-3-CT (3 nmol kg^-1), DP-3-CT (0.3, 3 nmol kg^-1), 8-OH-DPAT (40, 120 nmol kg^-1) and saline were given by microinjection (5μl over 20μl) i.c.v. 5-HT, DP-3-CT and 5-CT all caused rises in BP, HR and RNA of 24 ± 3 mmHg, 63 ± 13 beats min^-1, 126 ± 33 % and 24 ± 6 mmHg, 63 ± 9 beats min^-1, 112 ± 29 % for the highest dose of 5-HT and DP-3-CT respectively, and 44 ± 4 beats min^-1, 142 ± 43 % for 3 CT. 8-OH-DPAT 40 and 120 nmol kg^-1 only caused a rise in HR of 284 ± 30 and 13 ± 3 beats min^-1 respectively. 5-HT differed from DP-3-CT and 5-CT in that the rise in blood pressure was associated with an initial decrease in HR and RNA. PNA was only increase by 8-OH-DPAT. In rats pretreated with metyrapone attenuated the effects of DP-3-CT on all variables.

These results suggest that activation of 5-HT receptors are responsible for the sympathoexcitation and pressor effects observed when 5-HT is injected i.c.v.

397.16  

Microstimulation of the rat posterior insular cortex in phase with the ECG R wave elicits pure cardiac effects and has demonstrated insular cardiac chronotropic organization (Oppenheimer and Cechetto, 1991). As the latency to baroreceptor activation (SBHA) mechanisms mediate insular pressor effects (Cechetto and Chen, 1990), insular tachycardia was anticipated to be responsive to LHA manipulations. Insular tachycardia sites were physiologically stimulated once each cardiac cycle with 500 μA for 1 minute before and after microinjections into the LHA. The insular tachycardia response was abolished in chloralose-anesthetized rats by LHA microinjection of the synaptic blocker picrotoxin (250nm) attenuated tachycardia by 5% and LHA microinjection of kynurenine (250nm) attenuated the magnitude of the tachycardia response. We suggest that the LHA contains an obligatory synaptic mediator of insular tachycardia and that glutamate is the major neurotransmitter at this site. Neuromodulation of insular tachycardia may be affected by opiates and NPY receptors, a finding of considerable clinical relevance. (Supported by the Heart and Stroke Foundation of Ontario).
CARDIOVASCULAR REGULATION: FOREBRAIN MECHANISMS AND STRESS

NEURAL REGULATION OF "PSYCHOGENIC" ERECTION: HYPOGASTRIC NERVE SECTION.

Sixty male weaning rats of the Sprague-Dawley strain were divided into four groups. Two groups, exposed to illumination and darkness for three hours, were divided into two subgroups and housed in a standard 12-hour light/dark room. One group in each room was fed a purified diet containing 2% copper (Cu) while the other two groups were fed the same diet containing 1% Cu. This diet (wt/wt) (45/34, 24/37, 10/24) was based on sucrose (62%), egg white (30%) and corn oil (10%); it contains all known essential nutrients. The rats were killed after six weeks. Cholesterol measured by an enzymatic colorimetric method was elevated approximately 11% (p < 0.025) in the random light/dark cycle groups. This elevation was less than that (33%, p < 0.005) found in a preliminary experiment done with only the lower Cu diet. The lower Cu diet depressed both hematocrit (p < 0.004) and body weight (p < 0.009) approximately 6%. There was no anemia (mean hematocrit > 49%); a diet marginal in Cu may decrease body size and hematocrit. The results suggest shift workers are at risk for elevated plasma cholesterol.

IDENTIFICATION OF ANGIOTENSINERGIC PATHWAYS IN THE PVN.

Previous studies have indicated that the paraventricular nucleus (PVN) is important in the regulation of body water balance and cardiovascular activity. The PVN receives afferent input from the subfornical organ (SFO) (Lind et al., 1985; Gutman et al., 1986) and is essential in the expression of many components of the stress response including corticosterone secretion, autonomic activity, and increases in agonistic and locomotor behavior. The present experiment was designed to determine the degree to which water stress is generalized in BNST by determining if BNST neurons would respond similarly to two different stress-related stimuli. In acute experiments, 10 male Sprague-Dawley rats (150-350 g) were anesthetized with 1.2% ketamine/urethane. A bipolar stimulating electrode was placed in the amygdala and a recording glass microelectrode was placed in BNST. Eighty-four spontaneously active neurons were recorded from BNST. 66% of these cells responded to electrical stimulation of the amygdala, predominantly with driven activity (latency = 17.25 ± 5.41 ms). 33% responded to a noxious stimulus, tail pinch (TP), predominantly with an increase in firing rate, but only 4% responded to cutaneous stroke, a control somatosensory stimulus. A chi-square test of the interaction between amygdala stimulation and TP revealed that the proportion of neurons responsive to each is independent of one another (p > 0.25). Thus, while the neurons of BNST are responsive to at least two different stress-related stimuli, the pathways by which these reach BNST appear to differ.

REGULATION OF AUTONOMIC FUNCTIONS: GENITO-URINARY CONTROL

NEURAL REGULATION OF "PSYCHOGENIC" ERECTION:

EXPOSURE TO RECEPTIVE FEMALES FACILITATES REFLEXIVE ERECTION IN RATS, EVEN AFTER HYPOGASTRIC NERVE SECTION.

SIXTY MALE WEANLING RATS OF THE SPRAGUE-DAWLEY STRAIN WERE DIVIDED INTO FOUR GROUPS. TWO GROUPS, EXPOSED TO ILLUMINATION AND DARKNESS FOR THREE HOURS, WERE DIVIDED INTO TWO SUBGROUPS AND HOUSED IN A STANDARD 12-HOUR LIGHT/DARK ROOM. ONE GROUP IN EACH ROOM WAS FED A PURIFIED DIET CONTAINING 2% COPPER (Cu) WHILE THE OTHER TWO GROUPS WERE FED THE SAME DIET CONTAINING 1% Cu. THIS DIET (wt/wt) (45/34, 24/37, 10/24) WAS BASED ON SUCROSE (62%), EGG WHITE (30%) AND CORN OIL (10%); IT CONTAINS ALL KNOWN ESSENTIAL NUTRIENTS. THE RATS WERE KILLED AFTER SIX WEEKS. CHOLESTEROL MEASURED BY AN ENZYMATIC COLORIMETRIC METHOD WAS ELEVATED APPROXIMATELY 11% (P < 0.025) IN THE RANDOM LIGHT/DARK CYCLE GROUPS. THIS ELEVATION WAS LESS THAN THAT (33%, P < 0.005) FOUND IN A PRELIMINARY EXPERIMENT DONE WITH ONLY THE LOWER Cu DIET. THE LOWER Cu DIET DEPRESSED BOTH HEMATOCRIT (P < 0.004) AND BODY WEIGHT (P < 0.009) APPROXIMATELY 6%. THERE WAS NO ANEMIA (MEAN HEMATOCRIT > 49%); A DIET MARGINAL IN Cu MAY DECREASE BODY SIZE AND HEMATOCRIT. THE RESULTS SUGGEST SHIFT WORKERS ARE AT RISK FOR ELEVATED PLASMA CHOLESTEROL.

IDENTIFICATION OF ANGIOTENSINERGIC PATHWAYS IN THE PVN.


PHYSIOLOGY OF MUSCLE AFFERENTS IN THE BULBOSPONGIOSUS AND ISCHIOCAVERNOSUS OF RATS.

THE BULBOSPONGIOSUS AND ISCHIOCAVERNOSUS ARE PERINEAL MUSCLES THAT HAVE A ROLE IN RODENT MALE SEXUAL BEHAVIOR, SPECIFICALLY FUNCTIONING IN PENILE GAINST ENGORGEMENT (CUPS), DORSAL PENILE MOVEMENTS (FLIPS), AND EJACULATION. IT HAS BEEN SUGGESTED THAT SENSORY RECEPTORS IN THESE MUSCLES PLAY A ROLE IN THESE EVENTS AND POSSIBLY ALSO MEDIANE SENSATIONS ASSOCIATED WITH SEXUAL CLIMAX. TO INVESTIGATE THE PHYSIOLOGICAL RESPONSES OF THESE AFFERENTS, WE RECORDED FROM SINGLE AFFERENT FIBERS IN URETHRAL ANESTHETIZED RATS. SINGLE FIBER RECORDINGS WERE MADE FROM EITHER DORSAL ROOT FILAMENTS OR FROM THE MOTOR BRANCH (MB) OF THE PUDENDAL NERVE. THE MUSCLES WERE EXPOSED FOR STIMULATION. RECEPTORS WERE FOUND IN THE ISCHIOCAVERNOSUS MUSCLE AND IN ALL PARTS OF THE BULBOSPONGIOSUS MUSCLE. CONDUCTION VELOCITY MEASUREMENTS INDICATED THEY WERE PRIMARILY GROUP II MYELINATED AFFERENT FIBERS. ALMOST ALL OF THE AFFERENTS WERE TYPICAL MUSCLE STRETCH RECEPTORS EXHIBITING A RELEASING DISCHARGE, A REGULAR SLOWLY ADAPTING DISCHARGE RATE, AN INCREASED DISCHARGE TO MUSCLE STRETCH, AND A SHORT SILENT PERIOD DURING MUSCLE CONTRACTION. THEY ALSO APPEARED TO DIFFER IN PEPTIDE CONTENT AND PERINEAL REFLEX TEST. SOME AFFERENTS WERE RAPIDLY ADAPTING, LOCATED IN TENDINOUS TISSUE, AND INCREASED THEIR DISCHARGE DURING MUSCLE CONTRACTION. WE HAVE RECORDED FROM DORSAL PENIS NERVES DRIVEN BY MB AFFERENTS. THESE RESULTS SUGGEST THAT SENSORY RECEPTORS IN THE ISCHIOCAVERNOSUS AND BULBOSPONGIOSUS MUSCLES ARE ACTIVATED DURING PENILE EVENTS AND MAY PLAY A ROLE IN THE SEXUAL CONTROL OF SEXUAL BEHAVIOR. SUPPORTED BY NS27511.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
398.3 AUTONOMIC NEURONS ACQUIRE INNERVATION IN THE ABSENCE OF PREGANGLIONIC INPUT: A STUDY USING SYNAPsin AND MET-ENKEPHalin IMMUNOCYTOCHEMISTRY. N.M. Minorsky and W.G. Dall, Anatomy Dept., Sch. of Med., Univ. of New Mexico, Albuquerque, New Mexico 87131.

Neuronal Ganglia of the Major Pelvic Ganglion (MPG) supply autonomic innervation to the corpora cavernosa penis in the rat. Previous studies have shown that penile neurons receive the majority of their presynaptic input from preganglionic fibers which are immunoreactive for met-enkephalin (mENK). Whereas mENK innervation of penile neurons is greatly reduced following transection of the MPG, it is partially recovered in chronically decentralized ganglia. In order to determine whether other fiber populations contribute to penile neuron reinnervation, decentralized nerve terminals were immunolabelled with antibodies to synapsin as well as mENK. As expected, the level of synapsin staining was greatly reduced in the acutely decentralized ganglia and the number of innervated neurons was comparable to that seen by mENK immunocytochemistry (approximately 12%). In the chronically decentralized ganglia however, approximately 60% of the penile neurons were innervated by synapsin-IR fibers, compared to 30% described by mENK immunocytochemistry. These findings demonstrate a remarkable capacity for penile neurons to receive innervation in the absence of preganglionic input. This potential may be important for understanding autonomic control of pelvic visceral tissue following spinal cord injury.

398.5 PROJECTIONS OF THE ROSTRAL MPG: A REGION INVOLVED IN MODULATION OF SEXUAL FUNCTION. A.M. Peternel, L. Marson and K.E. McKenna. Northwestern University Medical School, Chicago, IL 60611.

The rostral nucleus paragigantocellularis (nPGi) modulates the tonic inhibition of sexual reflexes (Marson & McKenna, 1990). Mapping the projections to and from the nPGi will provide a neuroanatomical basis for understanding these reflexes. The anterograde tracer PHA-L, retrograde fluorescent tracers rhodamine and Fluorogold, and the anterograde/retrograde tracer HRP-WGA were microinjected into the rostral nPGi. Rats were perfused with a fixative appropriate for the tracing technique and sections through the brainstem and spinal cord (T13-S1) were processed. Anterogradely labelled fibers and terminals were found mainly ipsilateral at L5-L6 in the region of the medial motor neurons of the urethral sphincter, as well as in the lateral portion of this nucleus, near the motoneurons of the icchiovascular musculature. Fibers were also labelled in the DM nucleus at L5-L6. The motor and the bulbospongiosus are interspersed within this fibers. Fibers were also labelled in the IML and CA of T13-L2, the region of the lateral motoneurons of L4, and the sacral IML. Retrogradely labelled cells were found mainly contralateral in lamina X of T13-L3, lamina IV and V of T13-L1, and lamina IV of L2. Cells were also found in laminae IV, VIII, and of L4-L5 and in the medial gray and lamina IV of L6. Cells were also found in the laminae IV, V, and X of S1 and the ventral gray of S2. Anterogradely labelled fibers were found in medullary nuclei including the contralateral nPGi, NTS, nC1, facial nucleus and nucleus medianus reticular formation. In addition, fibers were found in the raphe, LC and subLC.


In d-tubocurarine pretreated rats electrical stimulation (0.5-15 Hz) of the central end of an acutely transected penile nerve or stimulation of the urethra at the level of the external urethral sphincter produced discharges (mean latency 13.8±2.4) on postganglionic nerves (PBN) to the urinary bladder (UB). The responses were potentiated (20-80%) by repetitive stimulation (7-10 Hz) and by i.v. administration of DMPP (100 ug/kg, 30-60 sec duration) on tetramethyl-ammonium (50 ug/kg). Ganglionic blocking agents (hexamethonium, trimethaphan, i.v.) or ietrotoxin applied to the nerve abolished the responses. The responses were not eliminated by acute destruction of the lumbosacral spinal cord, or chronic (1 week) removal of the contralateral major pelvic ganglion or the contralateral urethral nerve. However, chronic transection (1 week) of the ipsilateral pelvic nerve eliminated the evoked discharges on the PBN. Electrical stimulation of the penile nerve (2-10 V, 7-10 Hz) and colchicine (100 ug) was injected into the fourth ventricle. Cats were perfused 24 hrs later with paraformaldehyde/acrolein fixative (3/0.5%). Spinal cord sections (20 um) were examined microscopically to identify site sections. Sections of the medulla (40 um) were processed for 5-HT and THF or 5-HT and SP or SP and TH or 5-HT and M-ENK. 5-HT containing neurons were located in raphé pallidus (RP), obscurus (RO) and magus (RM), and the ventral medullary reticular formation (VLM). In RP and RO 60-90% of cells containing 5-HT also contained both THF and SP. In VLM 36 and 71% of cells which contained 5-HT also contained SP and THF respectively. In addition, in these regions M-ENK was found exclusively in cells containing 5-HT. Retrograde labelling was identified in RO. NP and VM neurons with both central and cortical cell labelling also in L3/L4. Quantitative analysis of cell distribution was performed. In the medulla cells were labelled in the nucleus paragigantocellularis, parapyramidal region, A5, raphe and in the region of the subcoeruleus. Labelling was consistent with known anatomical organization of pelvic systems. This procedure is a valuable tool in examination of spinal cord circuitry and neural systems involved in sexual function.

398.7 CNS CELL GROUPS PROJECTING TO THE PENIS LABELLED WITH PSEUDORABIES VIRUS (PRV). L. Marson, K.B. Platt and K.E. McKenna. Northwestern University Medical School, Chicago, IL 60611.

Transneuronal retrograde cell body labelling in the spinal cord and brainstem was identified after injection of Bartha's K strain PRV into the penis of 25 rats. Two days later rats were perfused with 4% paraformaldehyde and the spinal cord, pelvic ganglia and brain removed. Sections of the spinal cord, ganglia and medulla were cut (30-50µm) and processed with swine polyclonal antibody to PRV and visualized with avidin-biotin HRP complex. Retrogradely labelled cells were found in a restricted region of the pelvic ganglia. Transneuronally labelled cells were found in sympathetic and parasympathetic preganglionic regions in T3-L2 and L5-L6 of the spinal cord, respectively. In addition, cells were found in intraneuronal areas through the spinal cord and brainstem. This study demonstrates that spino-projecting 5-HT neurons in the cat medulla also contain THF, SP and M-ENK. (Supported by the NSF).


Previous studies from this laboratory have shown that MK-801, a non-competitive NMDA receptor antagonist, inhibits rhythmic bladder activity in the intact urethane-anesthetized (UA) rat, while having little effect in the decerebrate animal. The present study extends these initial observations by (1) examining the effects of MK-801 on bladder cystometrograms (CMG) and external urethral sphincter (EUS) EMG (electromyogram) activity, (2) compare MK-801 to CNXQ, an AMPA receptor antagonist, (3) determine the effects of UA on MK-801 inhibition of the micturition reflex. In UA (1.2 g/kg) intact rats MK-801 (0.003 mg/kg, iv) reduced bladder contractions in a dose dependent fashion, with EUS being somewhat more sensitive than the bladder. In decerebrate rats, MK-801 (0.003-3 mg/kg, iv) had little effect on bladder amplitude but increased EUS activity and reduced EMG activity by 70%. The effects of MK-801 (0.1 mg/kg, iv) administered to UA (0.6 g/kg) decerebrate rats reduced bladder contractions in a dose dependent fashion as with intact animals. CNXQ (0.2 mg/kg, iv) had no effect on bladder activity in either the intact, UA or the decerebrate rat.

Three conclusions are suggested by these data: (1) EUS reflexes are more sensitive to the effects of MK-801, (2) the AMPA glutamate receptor does not play a major role in micturition, (3) urethane enhances the effect of MK-801 on the micturition reflex in the rat.
398.9
BLADDER AND EXTERNAL URETHRAL SPHINCTER ACTIVITY EVOKED BY MICROSTIMULATION OF THE SACRAL SPINAL CORD.

Our previous studies demonstrate that microstimulation of the sacral spinal cord (SSC) produces robust external urethral sphincter (EUS) activity (1). We investigated the effects of microstimulation of the cruciate fossa (CF) and dorsolateral column (DLC) on EUS activity in anaesthetized cats using a microdrive with stimulation sites at 0.1 mm intervals. CF stimulation was performed in 33 cats, DLC stimulation in 11 cats, and combined CF and DLC stimulation in 11 cats. CF stimulation evoked EUS activity in all cats, with the maximal EUS activity occurring at 0.9 Hz. DLC stimulation evoked EUS activity in 9 of 11 cats, with the maximal EUS activity occurring at 0.7 Hz. Combined CF and DLC stimulation evoked EUS activity in all 11 cats, with the maximal EUS activity occurring at 0.9 Hz. These data indicate that stimulation of the cruciate fossa and dorsolateral column can evoke EUS activity, with the maximal EUS activity occurring at 0.9 Hz. These data suggest that stimulation of the cruciate fossa and dorsolateral column can be used to study the neural control of the external urethral sphincter.

398.10
EFFECTS OF MK-801, NPC 12626 AND HALOPERIDOL ON MICTURITION IN AWAKE AND ANESTHETIZED RATS.
P.L. Vera & M. Nahtaft. VA Medical Center, University of Pittsburgh, PA 15261.

We investigated the effects of MK-801, NPC 12626, and haloperidol on micturition in awake and anesthetized rats. We used chronic intravesical catheters in rats under halothane anesthesia to measure bladder capacity and micturition frequency. We observed that MK-801, NPC 12626, and haloperidol decreased the micturition frequency and increased the micturition volume in awake rats. We also observed that MK-801, NPC 12626, and haloperidol decreased the micturition frequency and increased the micturition volume in anesthetized rats. These data suggest that MK-801, NPC 12626, and haloperidol can be used to study the neural control of micturition.

398.11

We investigated the effects of SCI on the neural control of the bladder and rectum in rats. We used chronic intravesical and intrarectal catheters and recorded bladder and rectal EMG activity in awake and anesthetized rats. We observed that SCI decreased the frequency and increased the amplitude of bladder EMG activity. We also observed that SCI decreased the frequency and increased the amplitude of rectal EMG activity. These data suggest that SCI can be used to study the neural control of the bladder and rectum.

398.12

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398.13

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398.14

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399.9
REGULATION OF AUTONOMIC FUNCTIONS: GENITO-URINARY CONTROL

The neuronal hypertrophy of the preganglionic (PG) neurons (~ 800/μm²) and stimuli of 50-150 μm) and (3) near the PG axons along the lateral edge of the ventral horn (~2000 μm). The EUS responses were large during stimulation at the cord surface and diminished at the level of the PG neurons and axons. Transsection of the spinal cord (T5), of dorsal roots (S1-S2) bilaterally and contralateral VRs (S1-S3) did not change BC. However, ipsilateral VR (S2-S3) section abolished the BC. These studies suggest that BC can be evoked by microstimulation of the cord in the absence of major descending and afferent input and that BC does not reduce contralatal effrent outflow (Supported by NIH contract N01-NS-9-2366)

399.10
EFFECTS OF MK-801, NPC 12626 AND HALOPERIDOL ON MICTURITION IN AWAKE AND ANESTHETIZED RATS.
P.L. Vera & M. Nahtaft. VA Medical Center, University of Pittsburgh, PA 15261.

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399.13

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399.14

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The role of spinal serotonin (5-HT) and noradrenaline (NA) in micturition was assessed using neurotoxins and acute antagonists. Cystometrograms were performed in awake cats with chronic indwelling i.t. and bladder cannulas. Parameters followed were: volume threshold for micturition (Vt), postvoid residual volume, voiding contraction time and bladder stiffness during filling. Immunohistochemistry for tyrosine hydroxylase (TH) and 5-HT was performed to verify toxin actions. Methoxyflurane (2.5-20ug i.pl) and 5,7-DHT (1500ug i.pl) depleted TH and 5-HT in the lumbosacral cord and reduced Vt. Other parameters were unaffected. Prazosin (100μg i.v) was ineffective, suggesting that alpha-adrenoceptors are not involved in normal micturition (cf. Yoshimura et al, J Urol 139: 423, 1988).

SOMATIC AND VISCERAL AFFERENTS: CENTRAL PROJECTIONS


Autoradiography of muscarinic receptors (μ rec.) by [3H]DMAMGO was performed in laminae I-II at cervical levels following unilateral dorsal rhizotomies of 1, 3, 5, or 7 roots (R) around C7, at L1, T1, T10 and T12 days post-rhizotomy. Following 7R alone, a 37% decrease of binding was observed in the rhizotomized side compared to the intact side of the C7 segment. Sectioning up to 3 and 7 roots increased the μ recovery to a maximum of 70%. The residual binding is attributed to the postsynaptic component of μ rec. (Besse et al. Brain Res. 521, 1990) which remains stable over 3 months. From 15 to 90 days PL, after cuts of 1 to 5 roots, a "recovery" of binding occurs which is maximal when only C7 is cut. Because the more deprived the C7 segment is of affrents, the less pronounced is the recovery, the recovery is attributed to the presynaptic μ rec. This neuroplasticity indirectly suggests the occurrence of collateral sprouting of fine primary affrent fibers because μ rec. are essentially located on these fibers.

ULTRASTRUCTURAL CHANGES IN TERMINALS IN THE DORSAL COLUMN NUCLEI OF DIABETIC RATS: E. Andrade, I. Craig, S. Marom, and F. Tripp, Dept. of Pathology, East Carolina University, Greenville, NC 27858.

The ultrastructure of terminals in the gracile and cuneate nuclei of streptozotocin (STZ) diabetic rats were examined at 1.5, 10 and 30 days after the onset of diabetes. Terminals in the gracile nucleus were observed to be restricted to the polymodal morphology characteristic of neuropathic dystrophy in all experimental groups. Only the 30 day diabetic group showed dystrophic terminals in the cuneate nucleus. The dystrophic response was characterized by the decrease in population of synaptic vesicles, the presence and increased accumulation of tubulovesicular smooth endoplasmic reticulum, the increased presence of various forms of cellular debris, and the decrease of synaptic complexes. The proliferation of glial processes was evident in all groups but most notable in the 14 and 30 day groups. Occasionally, terminals which appeared dystrophic were observed in normal and control animals. These findings show that severe diabetes as modeled by the STZ diabetic rat has an early and progressive dystrophic affect on terminals in a somatosensory nucleus of the medullary dorsal horn system. Many of the dystrophic terminals had a morphology and synaptic configuration similar to primary affrents of the dorsal column nuclei. These observations are in agreement with clinical and experimental findings which often show the initial impairment of long sensory fibers and introduces the concept that the impairment of central transmission may be a contributing factor in the sensory deficits seen in diabetic neuropathy.


Intra-axonally labeled A High Threshold Mechanoreceptors (HTM) terminals identified physiologically had boutons which resembled the Dense Sinusoid Afferents (DSA) previously described in monkey lamina I and II. Using colloidial gold post-embedding immunocytochemical techniques we identified a new immunoreactivity (IR) in some dense-core vesicles found in DSA terminals, but we were not able to unequivocally demonstrate CGRP or SP in A HTM terminals. GABA-IR was found in vesicle containing profiles that were presynaptic to A HTM terminals and to other non-identified DSAs. CGRP and SP-IR were predominantly located inside presumed pre-synaptic profiles which contain numerous large dense-core vesicles (LDCV). GABA-IR profiles were in position to LDCV-containing terminals but synaptic contacts were rare. The terminals of two physiologically identified C fibers also contained GABA-IR dense-core vesicles. One C-fiber was a polymodal nociceptor. Supported by grants R01NS26340, NS16433 and NS14014899 from NIH.

CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVITY IN THE CAT SACRACOAL SPINAL CORD: C.R. Murray and L.A. Ritz. Dept. of Neuroscience and Neurosurgery, Univ. of Florida College of Medicine, Gainesville, Fl. 32610.

Our laboratory has been investigating the anatomical, physiological and behavioral organization of the cat sacral spinal cord, the portion of the neuraxis that innervates the urogenital tract. Recently, we have been concerned with the distribution of primary afferent fibers that exhibit CGRP immunoreactivity. CGRP labeling was found, as expected, within the superficial dorsal horn. A dense band formed across the entire mediolateral extent of the superficial portion of the dorsal horn. Labeling was also observed in an extensive mediolateral distribution at the level of the dorsal commissure. The apparent greater distribution of CGRP within the deeper portions of the sacrococcygeal dorsal horn than in other regions of the spinal cord is seen. Research supported by grant NS27511.

SOMATIC AND VISCERAL AFFERENTS: CENTRAL PROJECTIONS


The effect of 8-OH-DPAT, a selective 5-HT\(_A\) receptor agonist, on SMR has been investigated in urethane-anesthetized rats. Intravenous administration of 8-OH-DPAT (3.3 μg/kg) induced activation of SMR (bladder contractions > 16 mm Hg) in 67% of rats which bladders were filled with a volume just below that required for evoking SMR. The effect of 8-OH-DPAT was completely abolished by guanethidine and the MED was shifted to 330 ng/rat by 5,7-DHT administered iv in presence of desipramine. The activation of SMR by it 8-OH-DPAT was prevented by guanethidine, but was unaffected after 5,7-DHT iv administration. These findings indicate that stimulation of 5-HT\(_A\) receptor facilitate micturition reflex at both spinal and supraspinal sites. In both cases, removal of sympathetic inhibitory mechanisms prevented the action of 8-OH-DPAT suggesting that suppression of sympathetic outflow to the bladder could be involved in the effect of 8-OH-DPAT. In addition, 5-HT neurons seem to be important in the facilitatory action of 8-OH-DPAT on the SMR at supraspinal level.

REGULATION OF AUTONOMIC FUNCTIONS: GENITO-URINARY CONTROL

1003

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
399.5

The pelvic (PN) and pudendal (PUDN) nerves contain afferent fibers which are involved in sexual and excretory functions. The present study examined the central projections of these afferents by detecting C-FOS in spinal cord interneurons following unilateral electrical stimulation of the PN and PUDN in either desensitized or urethane (11.2 gm/kg) anesthetized cats. The PN and PUDN were stimulated at 2.5 times the threshold to produce bladder or perianal muscle contractions, respectively (usually 4-8 volts, 0.05 sec, 20 Hz, 10 sec on, 10 sec off, for 1 hour). One hour following the end of stimulation animals were perfused and tissue processed. PN stimulation produced the largest number of C-FOS positive cells in L5 and S1 segments, with few cells extending into L4 and S2. C-FOS positive neurons were found in the lateral lamina V at the base of the dorsal horn (DH), in the intermediolateral grey, in the superficial layers of the DH, and in the dorsal grey commissure (DGC). PUDN stimulation produced a wider distribution of C-FOS positive cells in L4-S5 with the densest labelling in L5-S5. The superficial lamina as well as laminae IV-VI and DGC contained positive neurons.

This study demonstrates similarities in the distribution of PN and PUDN afferent projections to the spinal cord and identifies possible interneuronal sites involved in the coordination of visceral and somatic components of urogenital function. (Supported by NIH contract NS-NS-8366)

399.6

Immunocytochemical experiments have shown that glutamate-positive neuronal somata in dorsal root ganglia are mostly small. Terminals of unmyelinated and small myelinated fibers in the superficial laminae of the spinal cord were also immunostained by the same glutamate antiserum. In the present experiments, we have investigated whether comparable good correlation exists with another class of primary afferent neurons and their terminals—those ascending in the dorsal columns and terminating in the dorsal column nuclei. At least 75% of terminals identified by anterograde tracing as endings of primary fibers ascending in the dorsal columns are glutamate-immunopositive. However, the majority of dorsal root ganglion neurons with an ascending branch to the dorsal column nuclei have large somata, and only 20 to 30% of projecting cells are glutamate-immunopositive. The difference in soma size between most dorsal root ganglion neurons that are glutamate-positive and those that project to the dorsal column nuclei suggests that glutamate may be present in cell bodies of large neurons of the dorsal root ganglia in a concentration insufficient to be detected with our present experimental conditions. This and alternative explanations for the apparent discrepancy are currently being explored.

399.7

In previous reports we have shown that terminals of dorsal root fibers remains to be established. in the DH, and in the dorsal grey commissure (DGC). PUDN stimulation produced a wider distribution of C-FOS positive cells in L4-S5 with the densest labelling in L5-S5. The superficial lamina as well as laminae IV-VI and DGC contained positive neurons.

Gene delivery, to alter the physiology of somatosensory neurons, may be possible using herpes simplex virus (HSV) type 1 vectors mutuated by insertion of DNA elements into the thymidine kinase (tk) gene. To achieve gene delivery to peripheral and central neurons, we tested thymidine kinase-negative (HSV tk) vectors containing the marker gene, lacZ, under the control of different mammalian and viral promoters. Regulatory elements included: HSV promoters classified as immediate early (hsp 9.5, early gene in RH116; both from Drs. Ho and Mocarski, Stanford Univ.), and hsp 6.5 (e.g. in p66: from Dr. Weinberg, and A. Rustioni, Depts. of Cell Biology & Anatomy and of Physiology, U. of North Carolina, Chapel Hill, NC 27599).

We have combined double-staining immunocytochemistry with anterograde transport to verify the simultaneous presence of elevated levels of both amino acids in primary afferent terminals. WGA-HRP was injected into the sciatic nerve of anesthetized rats. After 2-7 days, animals were perfused with 2.5% glutaraldehyde/0.5% paraformaldehyde/0.2% picric acid. Fifty mm Vibratome sections of L4 were cut, reacted for TMB with tumorstabilizer and embedded in plastic. Nickel mesh grids with thin sections were stained for glutamate and aspartate separately or in combination using different sizes of gold particles (Pendel et al., Neuosci. Abstr., 1996). Double staining for glutamate and aspartate was commonly observed among terminals of dorsal root afferents likely to be associated with unmyelinated and small myelinated primary afferents. A relation between these observations and the release of glutamate and aspartate from the same terminal of dorsal root fiber tracts may be established.

399.8
400.2
Effects of endogenous GABA on axonal conduction in the neonatal rat dorsal column.
Dept. of Neurosurgery, NYU Medical Center, New York, NY 10016
We have previously shown that GABA-A receptors activation can modulate axonal conduction in isolated dorsal columns of the neonatal rat spinal cord in vitro (Sakatani, et al. Brain Res., 542: 273-279, 1991). However, it is not known whether the GABA-A receptors in the dorsal columns of neonates are under physiological conditions. We consequently studied the effect of a GABA-upake inhibitor (sarcosine acid; NP) and a GABA-A antagonist (bicuculline; PTX) on axonal conduction in the dorsal columns, using whole monosynaptic (1.5-day-old) rat spinal cord (below T1) in vitro. The dorsal column was stimulated (0.5 msec, 2 mA, 0.2 Hz) by a bipolar platinum electrode placed 0.5 mm lateral to the dorsal root entry zone. Orthodromic dorsal column compound action potentials (CAPs) were recorded at two points with glass microelectrodes (1 MΩ).

NPA (1 mM) decreased CAP amplitudes by 12.5 ± 9.6 % of control (mean ± SD, n=6). This effect of NPA was blocked by PTX (1 mM). GABA at 0.1 mM mimicked the effect of NPA. However, the time course of NPA action was slower than that of GABA action, suggesting that NPA increased background extracellular GABA levels which would activate GABA-A receptors in the dorsal column. PTX (1 mM), alone, increased population conduction velocities, calculated from latencies of negative peaks, by 18.7 ± 3.2 % of control (n=3), suggesting that PTX blocks tonic endogenous GABA influences on the dorsal column axons.

These results suggest that pre-myelinated dorsal column axons of the neonatal rat spinal cord are tonically affected by endogenous GABA under physiological conditions. While the biological role of interaction of GABA and GABA-A receptors in the dorsal column is not apparent, these results support our hypothesis that axonal conduction in long tracts of the spinal cord is subject to GABAergic modulation. Supported in part by NIH grant NS1064.

400.4
Synaptic connections of functionally identified interneurons in hamster spinal cord.
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Department of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599-7545.

To investigate anatomical substrates underlying synaptic integration in the nucleus proprius (NP), we studied the ultrastructure of synaptic connections of NP interneurons in an isolated spinal cord preparation with intact sensory innervation.

NP interneurons were physiologically characterized and stained intracellularly with horseradish peroxidase (HRP). Terminal axon branches labeled with HRP were unmyelinated or thinly myelinated. Labeled synaptic boutons that were located in proximity to the parent cell body (i.e. within an area comparable to the spinal segment) were classified as spine to axon (160), spine to terminal (50), and terminal to terminal (40). Intracellular recordings were obtained with a GABA-A antagonist (bicuculline; 100 μM). However, it is not known whether the GABA-A receptors are under physiological conditions. We consequently studied the effect of a GABA-A antagonist (bicuculline; PTX) on axonal conduction in the dorsal columns, using whole monosynaptic (1.5-day-old) rat spinal cord (below T1) in vitro. The dorsal column was stimulated (0.5 msec, 2 mA, 0.2 Hz) by a bipolar platinum electrode placed 0.5 mm lateral to the dorsal root entry zone. Orthodromic dorsal column compound action potentials (CAPs) were recorded at two points with glass microelectrodes (1 MΩ).

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400.6
Synaptic activity of frog spinal dorsal horn neurons.
J.A. Medeiros, H. Tan and V. Mileic.

Dorsal horn neurons were studied in intracellular recordings from isolated hemisected spinal cords of adult frogs (R. pipiens). The average resting membrane potential was 68 ± 12 mV (n=118, range = -90 to -96 mV). The average membrane input resistance was 117 ± 68 MΩ (n=61, range = 20 to 340 MΩ). Preliminary experiments suggest that the neurons have a membrane time constant of approximately 20 msec. Spontaneous action potentials were observed in 20 of 160 neurons. These action potentials occurred tonically (18/20) or in bursts (2/20). Excitatory postsynaptic potentials (n=10), dorsal gray evoked potentials (n=7/160), and a combination of both (n=4/160) were observed to occur spontaneously.

Experiments were also conducted in which the dorsal root was stimulated by a suction electrode while the postsynaptic response was monitored in a dorsal horn neuron. The threshold for evoking a response in the dorsal horn neuron was 0.01 V (n=7; range = 5-4 to 424 μA). The average latency of response was 7 ± 11 msec (n=42; range = 0.5 to 68 msec). Compound postsynaptic potentials were observed in a few experiments. Preliminary experiments on the reversal potential suggest that the ionic conductances of the evoked postsynaptic potential are similar to those reported for mammalian dorsal horn neurons. (Supported by NIH NS1278).

400.5
Relative numbers of spinotubalar, supraspinal projection, propriospinalal and local circuit neurons in the superficial dorsal horn of the adult rat spinal cord.

The dorsal horn of spinal cord was studied in intracellular recordings from isolated hemisected spinal cords of adult frogs (R. pipiens). The average resting membrane potential was 68 ± 12 mV (n=118, range = -90 to -96 mV). The average membrane input resistance was 117 ± 68 MΩ (n=61, range = 20 to 340 MΩ). Preliminary experiments suggest that the neurons have a membrane time constant of approximately 20 msec. Spontaneous action potentials were observed in 20 of 160 neurons. These action potentials occurred tonically (18/20) or in bursts (2/20). Excitatory postsynaptic potentials (n=10), dorsal gray evoked potentials (n=7/160), and a combination of both (n=4/160) were observed to occur spontaneously.

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Whereas excitatory amino acids glutamate (Glu) and aspartate (Asp) have been implicated as neurotransmitters of the primary afferent fibers (PAF) the contribution of different categories of PAF to the release of Glu and Asp, and the regulation of this release by endogenous opioid peptides, the spinal level has not been characterized as yet. We have investigated whether the rate of outflow of Glu, Asp and glutaminase (Glu) from the spinal dorsal horn (DH) is modified in response to selected chemical activation of the small unmyelinated PAF by reserpine (RTX) and during the perfusion of the slice with a 5-opioid receptor agonist (D-Phe D-Phe)kynalphen (DPPDP). In superfused spinal cord slice - dorsal root ganglion (DRG) preparation, the DRG were superfused with RTX and the slice superfuse was collected and analyzed for amino acid content by HPLC with fluorometric detection. RTX (0.1-10 nM for 3-6 min) produced a prolonged (10-15 min), up to three-fold increase in the rate of basal outflow of Glu, Asp and Glu (n=6). Marked desensitization of the RTX effect was observed with the second RTX challenge. Preincubation of slices with DPPDP (10 μM, 12 min) effectively attenuated the RTX (2 nM)-evoked outflow of all the three amino acids. Supernusion with DPPDP resulted in a decreased rate of basal outflow of Glu and Asp in a proportion of slices (n=3). It is suggested that a population of small unmyelinated PAF may be an important neuronal source of released Glu and Asp in the spinal DH. A role for α-opioid receptors in modulation of somato-sensory processing at the spinal level is indicated. Supported by NS 36352 and BNS 841 804.


Serotonin acting at the 5HT receptor subtype may be involved in the modulation of somatosensory pathway function (Lai & Wicock, Soc. Neurosci. Abstr. 18. 97. 1990). In hatching anomalous tadpoles exogenous 5HT suppresses skin sensory activation of locomotor rhythmicity and we have explored the pharmacology and site of action of 5HT in mediating this effect. 5HT (0.5-5 μM) reversibly increases the stimulus threshold for activation of fictive swimming by up to 100%. Impulses in the primary afferents are not blocked by 5HT, indicating that its effects are mediated centrally. Intracellular recordings from postsynaptic sensory interneurones show that synaptic transmission is reversibly reduced by 5HT with no apparent change in conductance, suggesting a presynaptic site of action. The depression by 5HT is reduced by the specific 5HT3 receptor antagonist, MDL7222, but not by methysgeride or cyproheptadine. We conclude that in this simple developing vertebrate nervous system 5HT3 receptors are involved in the presynaptic inhibition of cutaneous sensory synaptic transmission. Supported by the Royal Society and SERC (UK).


Substance P (SP) has been proposed to play a regulatory role in nociception and both SP-like receptors are present in high quantities in the dorsal horn of the cord. However, direct evidence supporting this proposal is still lacking. Therefore a series of experiments was undertaken to examine this proposal. Sprague-Dawley rats were anesthetized in p. with Pentobarbitone (50 mg/kg) and chloral hydrate (50 mg/kg). After baseline readings were taken in the tail-flick test, one hind paw was immersed in water at 55°C for 1.5 min. Readings were taken at 3 min intervals for 20 min. For this study, binding studies, rats were decapitated at 1, 10 and 60 min following immersion. The spinal cords were removed and incubated in buffer containing [[H]BH-SP as described (Brain Res. 206, 295, 1990). In the physiological studies, immersion induced an increase in the reaction time of 150% in 1 min which gradually declined to 100% over 10 min. Binding of SP was depleted in the superficial parts of the dorsal horn of the lumbar cord in the 1 min group, with partial and full recovery in the 10 and 60 min groups, respectively. Binding around the central canal remained unaltered by immersion. These results indicate that noxious cutaneous stimulation to a hind foot induces an analgesia in the tail-flick test. Furthermore, the decreased [[H]BH-SP binding is most easily explained as being due to the increase in the concentration of NK-1 receptors by substance P. (Supported by grants from the Canadian MRC to JLH and RQ).


We sought evidence on whether ATP may be a synaptic transmitter for primary afferent fibres, by comparing actions of L-glutamate (Glu) and adenosine-5’-triphosphate (ATP) on substantia gelatinosa (SG) neurones in superfused 400-500 μm transverse slices of the spinal cord from young hamsters. Whole-cell patch recordings made with Cs+ or K+ filled electrodes. Both bath-applied Glu and ATP (0.4 to 3 mM) caused similar fast depolarizations and generated action potentials (AP) in 70% of neurones. ATP blocked the action potentials, but left fast Glu and ATP inward currents unaffected. All but one of 65 cells showing fast excitation by ATP also showed slow excitation by ATP; however, 10 of 54 neurones excited by Glu were unresponsive to ATP. ATP-γ-S, a non-hydrolysable ATP analogue, was as effective fast inward current while ADP and adenosine had no effect or were much less potent than ATP. A few neurones exhibited a prolonged depolarizing current to Glu after the initial fast component; a larger number showed prolonged inward current after ATP and ATP-γ-S. Since the SG receives most of its primary afferent input from thin fibres, these observations appear consistent with an ATP-like molecule acting as an excitatory synaptic transmitter for some of these fibres. (Supported by research grant HS-10321 from the NINDS.)

FACILITATION OF THE TAIL-FLICK REFLEX BY INTRATHecal ADMINISTRATION OF SUBSTANCE P AND BY NOXIOUS CUTANEOUS STIMULATION IS BLOCKED BY CP-96,345, A NONPEPTIDE SUBSTANCE P RECEPTOR ANTAGONIST. J. L. Henry, V. Radhakrishnan and K. Yashpal. Departments of Psychiatry and Physiology, McGill University, Montreal (Quebec)

Substance P has been proposed to regulate the excitability of nociceptive neurones in the dorsal horn of the spinal cord and to facilitate the nociceptive tail-flick reflex. Therefore, we examined the effects of CP-96,345 on facilitation of the tail-flick reflex 1) by intrathecal administration of 6.5 nmol of substance P at the lumbar spinal level in awake rats and 2) by noxious cutaneous stimulation in anaesthetized rats. In the latter experiment, rats were anesthetized by immersing the tip of the tail in water at 55°C for 1.5 min. Reaction time was decreased by about 70% by intrathecal administration of substance P and by about 40% by tail immersion. Systemic administration of CP-96,345 (0.5 I.V. or 5 s.c. mg/kg), but not of saline, blocked the facilitation induced by intrathecal administration of substance P and by tail immersion. However, neither CP-96,345 nor CSF altered baseline reaction time. These results indicate that while NK-1 receptors do not appear to be involved in mediating the tail flick reflex in either prepared intact or anaesthetized rats, they may be involved in expression of the facilitation of this reflex by substance P and/or by a related peptide. (Supported by Pfizer Central Research and by the Canadian MRC.)

RESPONSES OF DORSAL HORN NEURONES TO NOXIOUS CUTANEOUS STIMULATION AND TO ISOPTOTOPIC APPLICATION OF SUBSTANCE P ARE BLOCKED BY THE SELECTIVE, NONPEPTIDE SUBSTANCE P (NK-1) ANTAGONIST, CP-96,345. V. Radhakrishnan and J. L. Henry. Departments of Physiology & Psychiatry, McGill University, Montreal, Quebec.

Until recently, the known antagonists at the substance P (NK-1) receptor were all metabolically unstable peptides. Some of them were also known to exhibit agonistic and/or neurotropic properties. Recently, Snider et al. (Science 251:435, 1991) and McLean et al. (Science 251:437, 1991) reported a specific, nonpeptide NK-1 receptor antagonist, CP-96,345, devoid of agonistic property. We tested the effect of CP-96,345 on the responses of dorsal horn neurones to isoptotropic stimulation of substance P (which in electrophysiological studies selectively activates nociceptive neurones) and to noxious cutaneous stimuli. In chloralose-anesthetized cats, extracellular unit activity was recorded from neurones in segments L5-L7 using multibarreled microelectrodes. Neurones were first classified as either nociceptive (NK) neurones or to responses to natural stimuli to the skin. Some of the 8 neurones that responded to noxious thermal stimulus also responded to substance P. The slow excitation produced by substance P (0.5-120 nA), which lasted for 2-3 min, was effectively blocked by CP-96,345 (0.5 μg/kg, i.v.). CP-96,345 also attenuated the responses of noxious heat stimulus to these units, markedly reducing the after-discharge following stimulation. In the case of two substance P-insensitive units, the responses to noxious heat was unaffected by CP-96,345. CP-96,345 also inhibited the response of 2/4 units tested with noxious mechanical (pinch) stimulation. The results support the involvement of NK-1 receptors in the excitatory excitation induced in some dorsal horn nociceptive neurones by substance P and by noxious cutaneous stimuli. (Funded by Pfizer Central Research, the Canadian MRC and NIH.)
EFFECTS OF SLEEP ON SPINAL CORD DORSAL HORN LOW THRESHOLD NEURONAL ACTIVITY.

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The purpose of this study was to examine the effects of natural sleep on the response properties of spinal cord dorsal horn neurons to low intensity, repetitive electrical stimulation of their peripheral receptive fields (RFs). Extracellular recordings from primary afferents and dorsal horn neurons were obtained in each of 18 anesthetized, paralyzed, tracheostomized rats. Each rat was studied for four consecutive 1-hour recordings: one during the waking state, one during slow wave sleep, one during rapid eye movement sleep, and one during REM sleep. In the waking state, the response to electrical stimulation of the RF was characterized by an excitatory postsynaptic potential (EPSP). In slow wave sleep, the EPSPs were not observed. In rapid eye movement sleep, the EPSPs were of smaller amplitude and were associated with a slow-wave hyperpolarizing response. In REM sleep, the EPSPs were of larger amplitude and were associated with a slow-wave depolarizing response.

There was a significant increase in the EPSP amplitude and a significant decrease in the EPSP latency during REM sleep. The results of this study indicate that sleep plays an important role in the modulation of the response of spinal dorsal horn neurons to peripheral stimuli. The differences in the response properties of spinal dorsal horn neurons during the different sleep states suggest that sleep may play a role in the integration of sensory information.

Supported by NIH; YDK was funded by the FRSQ.

ASCENDING PROPRIOSPINAL CONNECTIONS TO THE UPPER CERVICAL SPINAL CORD IN THE RAT: POSSIBLE INVOLVEMENT IN FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY.

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It is well known that a large proportion of spinal neurons belong to a class of cells that interconnect different segments and levels of the spinal cord. Although these propriospinal neurons have been implicated in both sensory and motor function, little is known about the organization or function of the propriospinal system. The present study was carried out to evaluate the distribution of rat propriospinal neurons projecting to the upper cervical spinal cord and to study their potential involvement in the recovery of function following spinal cord injury.

Fluorescent tracers, including Fluorogold, DAPI and Fast Blue, were injected into cervical segments C2-C3; injection volumes ranged from 0.5-6.0 μl. Following survival times of 5-7 days animals were perfused transcranially with 4% paraformaldehyde, tissue was dissected and stained with DAPI fluorescence illumination. The distribution of neurons with ascending propriospinal connections included the dorsal and ventral horns, the region around the central canal, and the lateral spinal nuclei. Of the majority of labeled neurons were found within 5 segments of injection sites, large numbers of cells were also found throughout the lumbar spinal cord. In preliminary experiments, performance on sensory and motor tasks were assessed following mechanical or neurotoxic lesions in the thoracic spinal cord (T7-T12). In these animals functional recovery was found to correlate with the presence of propriospinal labeling from cord levels below the lesions to injection sites in the upper cervical cord. (Supported by NS28059 and The Miami Project).

A common feature of both clinical and experimental pain is the absence of habituation during prolonged exposure to noxious stimuli. The central nervous system mechanisms coding these prolonged sensations of pain, however, remain poorly understood. The present investigation examines the involvement of single spinal cord wide dynamic range (WDR) and nociceptive specific (NS) neurons to prolonged (20 minute) noxious thermal stimulation and relates them to psychophysical ratings of pain intensity. Spinal cord segments were transected in rats. Neurons with receptive fields included the distal portion of the hind limb. Stimulation consisted of cyclical immersion (5 sec on, 5 sec off) of the hindpaw into a heated water bath. The receptive field of each cell was stimulated with a neutral (35°C, 2.5 min) and one noxious temperature (45°C, 49°C, 47°C, 48°C, or 49°C; 20 min). Only one cell was examined per rat.

NS neurons exhibited a temporal response pattern dramatically different from that of WDR neurons. NS neurons stimulated with 49°C temperatures reached a peak and habituated to baseline (35°C) levels in less than 5 minutes. Discharge frequencies of WDR neurons similarly were highest during the first two minutes of stimulation, but importantly, failed to habituate substantially and were maintained at approximately 70% of peak levels during the subsequent 18 minutes of noxious stimulation. Psychophysical ratings of pain intensity closely paralleled the temporal pattern of WDR responses, indicating that WDR neurons, but not NS neurons subserved long-term thermal nociception. Supported by PHS grant NS-24000-1A2 and FIDIA Pharmaceuticals.


Prior work suggests that the circuitry needed to obtain conditioned antinociception extends to the level of the spinal cord. Because our past work used a reduced preparation (spinal) it is not clear whether this type of learning normally occurs intact in adult subjects. The present study addresses this issue by testing whether subjects that had been trained with the cord intact will exhibit conditioned antinociception after the cord has been transected. Rats were trained with intact cords receiving differential conditioning (which controls for sensitization and pseudoconditioning). One stimulus (the CS+) was paired with mild tail-shock (25 G, 0.5 mA), while the other (the CS-) was presented alone. Vibro-tactile stimuli to the left or right rear paw served as the CS+ and CS-. Which stimulus served as the CS+ was counterbalanced across subjects. The subjects received 30 CS+ and 30 CS- trials spaced 1 min apart. One hr after training all subjects received a spinal transection at T2. Twentyfour hrs later pain reactivity during the CSs was tested with the tail-flick test. Ten of the twelve subjects exhibited longer tail-flick latencies during the CS+ relative to the CS- (p < .05). These results suggest spinal systems can support associative learning in intact subjects. Supported by BNS B81981 to J.W.G.
401.5 THE RELATIONSHIP OF AN AVERSIVE OPERANT BEHAVIOR TO THE EXPRESSION OF C-FOS IN THE LUMBAR SPINAL CORD OF THE RAT. C.L. Lee and H.F. LEUNG. Divisions of Gastroenterology and A.B. Lee. Dept of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27599-7545. Expression of the proto-oncogene C-fos in spinal cord neurons was induced by administration of the skin, presumably through activation by primary afferents. This study corroborates the relationship of spinal afferent input to the expression of C-fos in the spinal cord. It was found that the pattern of C-fos expression in the spinal cord was influenced by the type of noxious stimulus used. Neurons in the superficial laminae of the spinal cord were found to be predominantly activated by noxious mechanical stimulation, whereas neurons in the deep laminae were activated by noxious thermal stimulation.

401.6 EFFECT OF SPINAL SOMATOSTATIN OR KAINIC ACID SUPERFUSION AND NOXIOUS SKIN STIMULATION ON IMMEDIATE EARLY GENE EXPRESSION IN RAT SPINAL DORSAL BORN NEURONS AT THE 3H14. F. Sandkühler. II. Physiologisches Institut, Universität Heidelberg, Germany.

Noxious skin stimulation causes rapid and temporal induction of immediate early genes such as c-fos and c-jun which may be released to acuteness nociceptor input to the spinal dorsal horn. To test whether somatostatin is involved in the induction of c-fos we have performed experiments on deep peritaneous anesthetized rats. Following laminectomy of l T4 and l T5 we superfused the dorsal horn cord by means of a specially prepared silicon rubber. The pool was filled with artificial CSF before and after superfusion with somatostatin (10 nM, for 30 min) or kainic acid (0.3 nM, 15 min) in one group of animals. In a second group the dorsal horn was superfused with artificial CSF alone for 1 hour. One hour after superfusion the c-fos mRNA levels were determined by in situ hybridization with a probe specific for c-fos. The results showed that the levels of C-fos mRNA were significantly reduced in the spinal cord of rats that were superfused with somatostatin compared to controls, suggesting that somatostatin may play a role in the modulation of c-fos expression in the spinal cord.
Conditioning or footshock itself, can activate endogenous
systems that have been paired with electric footshock during Pavlovian
conditioning. 

P. Roberson* & F.J. Helmstetter. Department of Psychology, 
Purdue University, West Lafayette, IN 47907.

The cortical potential evoked by electrical stimulation of the tooth pulp is
being evaluated as a nonbehavioral measure of antinociception in awake
animals. The potential is measured as the latency and amplitude of the P35,
a component of the mu rhythm, which is sensitive to behaviorally induced
nociception. In the present study, we investigated the inheritance pattern for
this insensitivity by measuring responsiveness to N2O in C57BL/6 mice and C57BL/6 parent strains, their B6D2F1, 
offspring, and B6/DYI recombinant inbred strains created by systemic ibmreeoding of an F1 cross of B6D2. N2O
antinociception was measured as percent suppression of acetic acid
abdominal contractions (AC) in 70% N2O compared to room air. 
C57BL/6 mice were highly sensitive to N2O (60% suppression of AC) and mice were intermediate in sensitiv-
ity (49% suppression). In 19 B6/DYI strains, responses to
N2O varied from 100% to 0% suppression: 

B6D14 -100%; 25 -100%; 22 - 95%; 05 - 94%; 28 - 95%; 31 - 89%; 01 - 89%; 24 - 88%; 12 - 86%; 09 - 82%; 
29 - 78%; 16 - 75%; 02 - 69%; 13- 62%; 32 - 61%; 
21 - 33%; 19 - 29%; 30 - 26%; 10 - 0%

This pattern suggests that multiple genes may be responsi-
ble for the underlying neurological anomaly conveying resistance to N2O antinociception. (Supported by N.I.H. 
Grants DE-06489 and grants from UIC COMOR and OB.

CORTICAL POTENTIALS EVOKED BY TOOTH PULP
STIMULATION TO STUDY PAIN IN AWAKE RATS. P.J. Danneman1, 
J. S. Zagon1, T. P. Stone2, S. Alper1, R. S. Zaslow1, R. B. Eisen1. 
Unit for Lab. Animal Medicine1 and 
Dept. of Neurology2, Physiology2, Univ. of Michigan Medical School, 
Ann Arbor, MI 48109 and Dept. of Neurosciences & Anatomy2, 
Penn State College of Medicine, Hershey, PA 17033

Behavioral tests to measure antinociception in animals are potentially
affected by the sedative as well as analgesic effects of drugs. Accordingly, 
the cortical potential evoked by electrical stimulation of the tooth pulp is
being evaluated as a nonbehavioral measure of antinociception in awake 
rats. Bipolar stainless steel electrodes are cemented into bur holes in the
dentin of the left maxillary incisor. Recording electrodes are attached to
shall screw implants over the cortex and a screw located over the cerebellum.
A screw located over the cerebellum is used as reference. The skull screws located over the contra- and ipsi-lateral somatosensory cortex
and vertex. A screw located over the cerebellum is used as reference. The
This protocol was approved by the Yale Animal Care and Use Committee. Male 
Sprague-Dawley rats (200-280g) were used. Both balloons were inserted intra-
ocy with local anesthesia. The P35 component was identified as the wave following the first deflection of the P wave. 

Male Sprague-Dawley rats (200-250g) were anesthetized by a single intraperitoneal injection of either 37.5 or 75.0 mg/kg P. Carrageenan (Cg; mg in 
150) was injected into the right hind paw footpad 15 min post P. Injection. At 1 hr and 18hr post Cg injection rats were anesthetized with pentobarbital and
the P35 component was measured. The P35 component was measured from the 
baseline to the first deflection of the P wave. 

Male Sprague-Dawley rats (200-250g) were anesthetized by a single intraperitoneal injection of either 37.5 or 75.0 mg/kg P. Carrageenan (Cg; mg in 
150) was injected into the right hind paw footpad 15 min post P Injection. At 1 hr and 18hr post Cg injection rats were anesthetized with pentobarbital and
the P35 component was measured. The P35 component was measured from the 
baseline to the first deflection of the P wave. 

The present study examined the effects of zolantidine 
(100-700 mg/kg, ip) on nociceptive 
activity (49% suppression). In 19 B6/DYI strains, responses to
N2O varied from 100% to 0% suppression: 

B6D14 -100%; 25 -100%; 22 - 95%; 05 - 94%; 28 - 95%; 31 - 89%; 01 - 89%; 24 - 88%; 12 - 86%; 09 - 82%; 
}
402.7
INHIBITION OF ANALGESIA FOLLOWING MORPHINE IN THE PERI-
AQUEUDUCTAL GRAY BY METHYSERGIDE IN THE VENTRAL MEDULLA OF
RATS. J. H. Kelly, M. L. Cooper and W. W. Goodman. Dept. of
Anesthesiology. Univ. of Kentucky, Lexington, KY.

Previous studies have demonstrated opioid and
nicotinic hyperalgesic and analgesic processes in the
dorsal posterior mesencephalic tegmentum (DMT) and
posterior medulla (PM) of the rat, respectively (FASEB J.,
1:8860, 1991). The hyperalgesic processes appear to
exhibit tonic activity since either naltrexone (N) or
meprylamine (M) produced dose related analgesia when
microinjected into the DMT. Using intact conscious rats
fitted with indwelling cannulae we have further explored
the pharmacology of these different brain stem regions.
In the DMT, three different local anesthetics [lidocaine
(L), bupivacaine (B) and cocaine (C)] produced prolongation
of low intensity thermally evoked tail avoidance reflex
(LITETAR). A selective kappa agonist, U-50,488, produced
dose related shortening of the LITETAR in the DMT which
was abolished by N. A selective SHIRIA agonist, 8-0H-DPAT,
duced prolongation of the LITETAR when administered in
the PM. These findings support previous hypotheses
including 1) presence of tonically active hyperalgesic
processes in the DMT and 2) presence of opioid kappa
hyperalgesic processes in the DMT. Further, the results
with 8-OH-DPAT indicate the presence of serotonin
analogues in the PM where opioid and nicotinic
analgesia has been previously demonstrated.

402.8
PHARMACOLOGICAL ASPECTS OF BRAIN STEM HYPERALGESIC AND
ANALGESIC PROCESSES IN THE RAT. S. R. Hamann, J. R. Holtman,
Jr., S. Parvisi, J. S. Fu and W. R. Martin. Dept. of
Anesthesiology. Univ. of Kentucky, Lexington, KY.

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402.9
INHIBITION OF PGI2 FORMATION PREVENTS THE EXCITATORY
ACTIONS OF BRADYKININ IN NODOSE NEURONS OF THE
GUINEA PIG IN VITRO. G. M. Koschorke, D. Weinreich, G. E.

Bradykinin (BK) increases neuronal excitability in a
subpopulation of nodose neurons by selectively blocking a
spike induced slow afterhyperpolarization (AHP) This effect is
dependent upon the production of a prostaglandin (PG) (Europ.
J. Pharmac., 132: 61-63, 1986). To identify which PG is
mediating this BK effect, PG release from individual nodose
ganglion was assayed using GC/MS analysis. Spontaneous
PG release ranged between 1 and 20 fmole/ganglion for PGD2, PGF2a,
PGF2, TxB2, and PGI2. Of these PGs, only PGI2
synthesis was significantly elevated by BK (100 nm) application,
from 24 ± 4 to 51 ± 6 fmole (n=5). Tranycypromine (TCP, 10
μM), a PGI2 synthetase inhibitor, had no significant effect on
spontaneous PG release but it completely abolished the BK-
induced increase in GL formation.

When PGD2, PGE2, PGF2, TxB2, or PGI2 were superfused onto
duo we onset ganglion neurons, only PGI2 blocked the AHP.
TCP (10 μM) did not effect the AHP but its presence in the
superfusate totally prevented the BK-induced block of the AHP.
(n=6). We conclude that BK blocks the AHP by selectively
stimulating the synthesis of PGI2. (NIH grant NS22069 to DW)

402.10
BRADYKININ STIMULATION OF DORSAL ROOT GANGLION
CELL CULTURES. M. B. Bauer, M. L. Simmons, S. Murphy &
G. F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa
City, IA 52242.

Although prostaglandins are traditionally considered to have
a peripheral involvement in nociception, they have been implicated
as also having a central role. To test for a central role of
prostaglandins in nociception, dorsal root ganglion cells were
cultured from embryonic 15 - 19 day old rats. After 12 days in
culture with Dulbecco's Modified Eagles Medium containing NGF
and mitotic inhibitors, the cells appeared to be predominantly
neuronal. Lomoxyn (10-5 M) or bradykinin (BK; 10-3 M)
stimulated the release of arachidonic acid (AA) metabolites from
[3H]AA-labelled cells. Specific RIA revealed that BK (10-6 to 10-3
M) stimulated the release of both TXA2 and PGD2 but did not
stimulate the release of TXA2. Additionally, it appeared that BK
(10-6 M) stimulated the release of guanylyl cyclase-activating
factor (GAF), the production of which was inhibited by NMA (N-
monomethyl arginine). Overall, it appears that BK stimulates
the release of prostacyclin and possibly a nitrosyl compound from
cultures of embryonic dorsal root ganglia.
RECORDINGS FROM DEPOLARIZING AMACRINE CELLS (ALL-LIKE) SHOW A TEMPORAL LOS OF THE LIGHT EVOKED RESPONSE IN THE RABBIT RETINA AND PHOTORECEPTORS: CIRCUITS AND SIGNALS

H. C. supported by a Warner Lambert postdoctoral fellowship.

NBQX REVEALS A PROMINENT NMDA RESPONSE IN THE RABBIT RETINA. H. C. supported by a Warner Lambert postdoctoral fellowship.

NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[F]quinoxaline) is the most selective antagonist yet developed for non-NMDA vs. NMDA excitatory amino acid transmission. We have studied NBQX in the superfused rabbit retina using two methods. First, we used the 2-deoxyglucose autoradiographic technique. All drug treatments were given daily for up to 9 days postsurgery. 1) Intraperitoneal (IP) administration of a low dose of morphine (1.5 mg/kg sc), 15 min before the swim sessions actually reduced the analgesia that resulted. The combination of morphine and cold water produced less analgesia than did either separately. A similar pattern of findings resulted from the administration of naloxone or morphine before another stressor that produces nonopioid SIA, social defeat. Intruder rats were exposed to established colonies for 10 min on 5 consecutive days. Naloxone or morphine was administered before the daily defeat session. Naloxone potentiated the resulting analgesia, and animals given defeat and morphine were less analgesic than those that received only morphine. These data provide further support to the idea that there is an interactive relationship between opioid and nonopioid analgesia systems. Supported by NSF grant BNS 88-09527.

MONOSialoganglioside GM1 REDUCES NOCICEPTIVE BEHAVIORS AND SPINAL CORD METABOLISM IN RATS WITH PERIPHERAL MONONEUROPATHY. L. Magi, R. L. Hong, D. D. Price, R. C. Caghill, J. Lu, and D. J. Mayer. Dept. of Physiology, 2Neurosurgery, and 3 Anesthesiology, Medical College of Virginia, Richmond, Virginia 23284

Effects of a monosialoganglioside GM1 were examined on nociceptive behaviors and spinal cord metabolic changes in rats with a peripheral mononeuropathy produced by sciatic nerve ligation. Behavioral changes of ligated rats were assessed by the radiant heat foot-withdrawal test and by rating spontaneous hind paw guarding positions. Effects of GM1 treatment on spinal cord metabolic activity were examined by using the 2-deoxyglucose autoradiographic technique. All drug treatments were given daily for up to 9 days postsurgery. 1) Intraperitoneal (IP) administration of a low dose of morphine (100 μg/kg, n=6/group) reduced hyperalgesia of the rat hind paw ipsilateral to nerve ligation, when initiated up to 24 hr after nerve ligation (ANOVA, F<0.01). 2) The hyperalgesic response also significantly decreased in a dose dependent manner in ligated rats when intrathecal (IT) GM1 was initially administered 1 hr after nerve ligation at the lumbar spinal cord (10 μmol, n=7/group), but not at the cervical spinal cord (20 nmol, n=7/group), indicating a lumbar spinal mechanism of GM1 action. Moreover, GM1 significantly lowered the rate of spontaneous hind paw guarding positions (P<0.05), suggesting the attenuation of nociceptive pain. 3) IF GM1 (10μg/kg, n=6) initiated 1 hr after nerve ligation significantly reduced the abnormal increase of metabolic activity seen 10 days following nerve ligation in all four sampled regions (laminae I-IV, V-VI, VII, VIII-IX) on both sides of spinal lumbar segments (L2-L5)(ANOVA, P< 0.01). This decrease of spinal cord neural activity may also reflect the influence of GM1 on spontaneous pain. Our data suggest that ganglioside treatment may be a promising approach for clinical management of neuropathic pain syndromes. Supported by Fidia Pharmaceuticals.

A rod in cat retina contacts 2 rod bipolar cells at a single ribbon synapse. The presynaptic membrane subjacent to the ribbon (arciform density) extends for almost 2 um, denoting an area over which ‘100 synaptic vesicles can attach to the ribbon. The ribbon surface serves as deposit for ‘100 vesicles. We reconstructed from electron micrographs of serial sections the corresponding synaptic apparatus at the second stage of the circuit where 2 bipolar axons contact 5 All amacrine cells. Individual ribbons in the rod bipolar axon are small: length of arciform density = 0.2 ± 0.09 um; surface area = 0.002 ± 0.025 um². However, the number of ribbons is: 59 ± 3 ribbons/2 bipolar axons. Consequently the total area for attachment at the presynaptic membrane can accommodate ~765 vesicles, and the total ribbon surface can accommodate ~1500 vesicles. Thus, the synaptic apparatus at the second stage expands by roughly 5-fold.

The signal in a rod due to photosensitization is prolonged (integration time ~250 ms), but at the All it quickly decreases to about 50 ms. If the total length of the arciform density is proportional to the number of vesicle release sites, and the probability of release is the same at each site, then the number of vesicles modulated by one photosensitization (release sites x probability of release x integration time) would be similar at both stages. Supported by EY00828.

403.5 TWO DIFFERENT EXCITATORY AMINO ACID RECEPTORS IN ON-BIPOLAR CELLS OF THE MUDPUPPY RETINA. W. B. Thompson and R. F. Miller. Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

Whole cell recordings were obtained from ON-bipolar cells in the mudpuppy retinal slice. At a holding potential of -50 mV, L-2-amino-3-phosphonobutyric acid (L-AP4,5-10 μM) produced an outward current similar to L-AP4. After washout of the L-AP4 response to 20 μM glutamate (GLU), a slow conductance decrease associated with desensitization was observed at concentrations up to 40 μM GLU. At concentrations above 40 μM, the initial peak response continued to grow, but the steady state response declined somewhat due to desensitization. 2) Cells with no response to 20 μM GLU, but a response to 20 μM L-AP4 resulted from the fact that 1) a less sensitive monoclonal antibody against enkephalin was used and 2) the double-label paradigm utilized a combination of a fluorescein- and peroxidase-label. For the present study, a more sensitive double-label paradigm was performed on cryosections collected throughout each of the four retinal quadrants. Primary antibodies were raised in different species, while respective secondary antibodies were conjugated to either fluorescein- or rhodamine-isothiocyanate. An examination of more than six thousand labelled cells in each of the retinal quadrants revealed that all labelled cells express both enkephalin- and neurotensin-immunoreactivity. Therefore, these results indicate the presence of a single population of chick amacrine cells in which each contains both of these putative neuropeptide activities.

This work was supported by NIH grant EY05822 and by the Retina Research Foundation (Iplousten).
304.9

CHANGES IN HORIZONTAL CELL TEMPORAL FREQUENCY SENSITIVITY RESULTING FROM STEADY ANGULAR ILLUMINATION. I.D. Thompson, M.P. Graham and A.J. Parker, University Lab. of Physiology, Oxford, OX1 3PT, U.K.

The ferret is increasingly used in visual neurobiology. We have characterized the spatial properties of the neurons in its visual cortex using drifting sinusoidal gratings. Pigmented ferrets were anaesthetized (70/30 N2O/O2, Sagatal 1 mg/kg/hr) and paralysed. Gratings were presented (luminance, 120 cd/m²; contrast, 0.84) and analysis of spike time and waveform was performed by BrainWavz software. Surface normal penetrations were made into cortex representing central visual fields. Hand plots of receptive fields showed orientation tuning and, in the upper layers, marked end-inhibition. When stimulated with drifting gratings, some neurons showed a modulated response; others were unmodulated. A range of acuities could be observed within a single penetration. In one case, there was a change from 1.4 c/dgr to 0.8 c/dgr for the same cell and, in another, from 1.4 c/dgr to 1.0 c/dgr. Orientation tuning to drifting sinusoidal gratings at peak spatial frequency yielded bandwidths at half-height that were typically around 60 degrees. Normally, adjacent neurons displayed similar peak orientation tuning but, in one pair of neurons recorded simultaneously, the peak orientations differed by 90 degrees. Supported by SERC (UK), MRC: UK) and McDonnell-Pew.

304.10

A MODEL OF RESPONSES OF CAT'S TYPE I AMACRINE CELLS INVOLVING PARALLEL INPUTS FROM ON AND OFF BIPOLAR CELLS. D. Naylor, D. Tranchina, R.C. Hup, Dept. of Biology, Mt. Sinai Sch. of Med., N.Y., N.Y., 10029.

The linear component of the type I cell response to white noise light input accounts for 85-95% of the power in the output of the Winer kernel contributes 6-10%. Thus, a successful model of high-frequency responses should incorporate two such kernel functions. We investigated the properties of the type I cell when driven by contrasted annulus. Our model for type I is an extension of the model for type C, which has been shown to account for the responses of type C cells well. The type I model exhibits many properties in common with type C, including a kinetics of the impulse response, a delay before the appearance of the impulse response, and an increase in impulse response over a range of input frequencies.

304.11

COMPARISONS OF VOLTAGE-GATED CURRENTS IN SECOND AND THIRD ORDER NEURONS IN THE SALAMANDER RETINA. I.D. Pan* and M.J. Slaughter, SUNY School of Medicine, Buffalo NY 14214

Whole cell voltage clamp experiments were performed on isolated cells from the tiger salamander retina to characterize and compare the excitable currents. Bipolar cells were found to have large delayed-potassium currents that could be blocked by tetraethyl ammonium (TEA), fast activating and inactivating potassium currents blocked by 4-amino pyridine (4-AP), and an inwardly rectifying current blocked by tetraethylbarium of potassium. Small inward calcium currents, both sustained and transient, but no sodium current, were observed. The principal voltage gated currents in the physiological range were calcium currents. Horizontal cells also had prominent delayed potassium currents, thought at about a tenth of the biphasic cell current density. The A-type current was found to be outwardly rectifying in current that could be blocked by TTX. Calcium current in horizontal cells was very small, possibly reflecting the calcium-independent release of transmitter. In contrast, third order neurons have very large inward sodium currents. An A-current is usually present, with faster kinetics than that in bipolar cells, and a large delayed potassium current was found that is similar in current density to bipolar cells. No inward rectifying currents were observed. The calcium currents, both sustained and transient, were much larger than those found in retinal neurons. Supported by NSF BNS8919993.

304.12

PHYSIOLOGICAL SUBLAMINATION OF THE INNER PLEXIFORM LAYER (IPL) IN THE TURTLE RETINA. I. Ammermüller, H. Kolb, R. Normans, R. Mellert, University of Oldenburg, Germany, and University of Utah, USA.

We intracellularly recorded and stained 50 amacrine and ganglion cells in eyecup preparations of Pseudemys turtle. We have identified 21 of the 29 amacrine cells in the IPL of the 21 ganglion cells described by Kolb (1982). This sample allows us a complete description of the physiological sublamination of the IPL by correlating the light responses of the identified amacrine cell types with their stratification levels in the IPL. Thus amacrine cells with processes in distal sublamina A (S1) respond with sustained hyperpolarizations and those in proximal sublamina B with sustained depolarizations. Transient responses are correlated with amacrine stratifying in S2/3 and S3 on the ON-OFF sublamina A/sublamina B border. Ganglion cells are either OFF-center when stratified from S5-S5-S3 are sustained OFF responses. Similarly, ganglion cells mono- or bistriated from S3-S5-S5 are sustained ON center. As expected, most bi- and tristratified ganglion cells with dendrites in both sublaminae respond in an ON-OFF fashion while diffuse ganglion cells with branches throughout the IPL gave ON center responses. With the majority of IPL branching neurons being multistratified in the turtle retina, the simple bisublamination of ON and OFF responses seen in cat retina is obviously not as clear cut. Nevertheless our results show that an ON-OFF subdivision is largely valid for amacrine cells and for ganglion cell types with dendrites restricted to one or other of the sublaminae.

305.2

RESPONSES OF CELLS IN RABBIT AREA 17 TO DRIFTING SINUSOIDAL GRATINGS. Motokochiwa, T., Donald, M.F., Robert, A., Casse, N.T.


As part of our continuing inquiry into spatial and temporal properties of neurones in rabbit area 17, we have recorded responses to drifting sinusoidal gratings. Single cell activity in anesthetized and paralyzed rabbits was recorded. Units were identified as simple (S) or complex (C) with light or dark bars and stationary spots. In simple cells (S, n=60), only 50% responded to gratings with modulated amplitudes. The remainder (50%) were unresponsive to the same stimuli. On the other hand, 85% of complex cells C (n=20) had an unmodulated response to sinusoidal gratings, and only 2 of them were unresponsive. In the other 2, the discharge was modulated at optimal frequency. Six other cells could not be classified as either S or C. A large proportion of both S and C cells, in particular those with strong spatial frequency preference, were unresponsive to gratings with modulated spatial frequencies. They responded only when presented with gratings of constant spatial frequency. From the data, it appears that the simple units are more sensitive to the temporal frequency of their stimulus. Supported by SPCAR, NSERC and MRC.
**404.3**


The goal of this study was to examine the extent to which the visual cortex neurons were influenced by neighboring cells in the anesthetized rabbit. Using single cell recordings, 84 striate units were evaluated for responses to both stationary (on/off) and moving light bars before and after injection of 300nl of either lidocaine or GABA 2mm away from the recorded cell. Surprisingly, responses to stationary spots were affected in 55% of the neurons, while the responses to moving spots were modified in only 36% of the cells (P<0.02; x2=7.5; df=2). When directional index (DI) was <50%, the on/off response was modified more often (P<0.01;x2=45.9) than in units whose DI was >50%. Amongst the non-directional neurons, simple cells showed mainly decreased responses (67.7%), while the complex cells were mostly increased (62.5%). In contrast, direction selective (DI>50%) simple and complex cells maintained unaffected on/off responses (66.6% vs 80%). Results suggest that the non-directional cells are more dependent on horizontal cortico-cortical connections and on/off responses are mediated through different mechanisms in simple and complex cells. Supported by an FCAR grant to S. Molotchnikoff and P. McKinley

**404.4**

**EFFECTS OF SUBSTANCE P IN THE CAT STRIATE CORTEX ARE SELECTIVE FOR CELL TYPE AND CORTICAL LAYER.** H. H. Chen1, T. P. Pickard1, and A. Albright2.


In the cat striate cortex Substance P (SP) was found to intrasynaptically projecting neurons of layers V and VI. The SP content is high during the first postnatal month and thereafter decreases dramatically (Wahle et al., Soc. Neurosci. Abstr., 14, 7455, 1988). We tested the effects of SP on one month and one year old cortex to seek for a functional role of SP in striate cortex and for possible differences in SP effects between immature and mature cortices. Drugs were delivered microiontophoretically during extracellular recording and visual stimulation in anesthetized and immobilized animals. SP affected about 20% of the cells tested (N=93). Its main effect was a reversible, strong enhancement of visually evoked responses (VER) and/or spontaneous activity. The affected cells either had complex receptive fields (layers V and VI) or gave sluggish responses to visual stimulation (deep layer VI or white matter). The enhancement commenced after some delay (1-3 min) and generally lasted for at least 2 min. Our findings suggest a role for SP in modulating synaptic transmission in cat visual cortex. The exact mechanism of this action will be elucidated by ongoing experiments.

**404.5**

**MICROIONTOPHREOTICALLY-APPLIED NORADRENALENE AND SEROTONIN HAVE FUNCTIONALLY ANTAGONISTIC EFFECTS ON DIRECTIONAL PROPERTIES OF COMPLEX CORTEX NEURONS.** J. Goyer and M.S. Cynader, Dept Ophthalmology, Univ. British Columbia.

The effects of microiontophoretic applications of noradrenaline (NA) and serotonin (5-HT) on response properties of visual cortex neurons were assessed in anesthetized, paralyzed cats. Unit responses to single flashes and bidirectional sweeps of bright slits of the optimal orientation, as well as orientation tuning curves were assessed before, during and after NA or 5-HT application. The signal-to-noise ratio of the activity evoked by single flashes (duration 400 msec) was significantly increased after NA application (16.8 vs 10.5), but was decreased after 5-HT application (4.6 vs 10.5). The direction selectivity of the assessed cells (as measured with bidirectional sweeps along the cell's preferred orientation) was also significantly increased by NA (7.8 vs 4.5), and decreased by 5-HT (1.9 vs 4.5). The sharpness of the orientation tuning curves was again increased by NA and decreased by 5-HT.

These results suggest that NA and 5-HT play functionally antagonistic, modulatory roles on the visual cortex responses to different stimuli, NA acting to "sharpen" the cortical cell's response, while 5-HT "smooths" cortical cell response.

**404.6**

**THE ROLE OF GABAERGIC INHIBITION IN THE ADAPTATION OF DIRECTION SELECTIVITY IN NEURONS IN AREA 17 OF THE CAT STRIATE CORTEX.** J. Goyer, M.S. Cynader, and Max S. Cynader, University of British Columbia, Dept. Ophthalmology & Psychology, Vancouver, B.C.

We investigated a possible mechanism for adaptation of simple cortical cells using iontophoretically applied GABA receptor agonists and antagonists. The prolonged unidirectional visual stimulation was compared to the adaptation during ionophoretic application of the GABA antagonists bicuculline (GABA-A) and phaclofen (GABA-B), as well as the agonists GABA and Baclofen. Bicuculline increased the firing level of all cells by approximately 100%. Both preferred and nonpreferred direction of motion responses increased. In a small number of cells, phaclofen increased the responses by about 33%. Neither bicuculline nor phaclofen blocked the adaptation to prolonged stimulation, and the higher responsiveness under bicuculline actually increased the rate of adaptation. GABA and Baclofen dramatically decreased the firing of cortical neurons, however, adaptation to prolonged stimulation was still observed. Adaptation-induced changes in the direction selectivity of the neurons were also observed during bicuculline and phaclofen application, even in cells where the nonpreferred direction motion responses had increased to equal the preferred direction responses. Bicuculline alone (without visual stimulation) dramatically increased spontaneous activity, but did not cause a adaptation-like reduction in the response rate suggesting that pre-synaptic deletion of neurotransmitter may be an important part of the mechanism of adaptation in cortical neurons.

**404.7**


The spatial organization of orientation tuning was revealed with the use of small oriented bar stimuli (Hohel & Wiesel). Recently optical imaging revealed that orientation tuning maps were obtained by large flashes produced functional maps identical to those produced with small bars. Thus, lateral interactions between activities evoked by multiple bars within the grating did not change the spatial maps.

Here we examine functional interactions among regions, tuned to different orientations, in area 17 of cat visual cortex. We employed moving plaids stimuli composed of two orthogonal gratings and extending over large visual angle (>15°). Using optical imaging based on intrinsic signals in anesthetized and paralyzed cats. Either moving plaids or their individual components were used as stimuli. To determine whether significant interaction exists among regions tuned to different orientations (activated simultaneously), we compared activation maps generated by the above stimuli set. We found that the maps generated by moving plaids stimuli were identical (in shape and intensity) to the sum of maps generated separately by its components. This finding indicates that, at the level of population activity, the interactions associated with moving plaids happen in a manner different from the summation of the activation of area 18. If perceptual direction of motion is spatially coded, plaids motion coding should produce a map which differs from a linear sum of its components. Thus, our results are consistent with a two stage model for motion processing: Area 17 stage codes local motion of oriented components (bounded by the "aperture problem") while the stage of motion integration is performed at higher visual areas.

(Supported by the Riky Family Foundation)
404.9 NON-LINEAR RESPONSES TO APPARENT MOTION IN CAT VISUAL CORTEX NEURONs. J.C. Boulton* and C.L. Baker, Jr., Dept. of Medical Physics, Utrecht University, The Netherlands, and Dept. of Psychology, McGill University, Montreal, Canada.

Recent human psychophysics has shown that the perception of movement from two-flash apparent motion of random Gabor kinematograms can be predicted from the stimulus spatiotemporal power spectrum only when the stimulus onset asynchrony (SOA) is less than 100 ms. For larger SOAs this relationship breaks down, implying nonlinearity (Boulton and Baker, ARVO 1991).

Comparable experiments on direction selective cells in Areas 17 and 18 of the visual cortex reveal similar behavior. Two-flash apparent motion of a Gabor stimulus was used to examine direction selectivity across a range of SOAs (0 to 200 ms) and jump sizes (0 to 1 cycle of the cell's optimal spatial frequency, lambda). For short SOAs (< 100 ms) linear behavior is observed with peak directional responses for small jump sizes (< 1/4 lambda), often followed by reversal for larger jump sizes. However, many cells also showed strong directionally selective responses at long SOAs (> 100 ms) and large jump sizes (> 1/2 lambda). These non-linear responses are compared to human psychophysical performance.

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404.10 SPATIAL ORGANIZATION OF INHIBITORY INFLUENCES UPON NEURONS IN THE CAT'S STRIATE CORTEX. J.C. DeAngelis*, J.G. Robson‡, L. Ohzawa, and R.D. Freeman, School of Optometry, Univ. of California, Berkeley, California 94720, and Physiological Laboratory, Cambridge CB2 3EG, U.K.

Intraretinal inhibitory interactions are known to be involved in the generation of receptive fields of neurons in the visual cortex. In addition, there are two main types of inhibitory influences which modulate the responsiveness of striate cortex neurons: end-inhibition (or endstopping) (Hubel & Wiesel 1965) and cross-orientation inhibition (Morrone et al. 1982). We have studied the spatial organization of these inhibitory influences through extracellular recordings of single unit responses from the striate cortex of anesthetized and paralyzed cats.

Length-response and width-response curves are constructed using rectangular patches of drifting sinusoidal grating centered on the receptive field. Cells which exhibit an optimum in their length-response curves (end-inhibition) tend to show a similar fit in their width-response curves (side-inhibition). Tests with superimposed gratings of different spatial frequencies suggest that regions eliciting end- and side-inhibition only slightly overlap the excitatory receptive field. In similar tests, superimposed gratings of orthogonal orientations are used to measure the spatial summation of cross-orientation inhibition. For all cells tested, the summation area for cross-orientation inhibition is centered upon the excitatory receptive field and of similar or smaller extent. Thus, cross-orientation inhibition is confined within the excitatory receptive field, while end- and side-inhibition arise from surrounding regions.

End- and side-inhibition may be further distinguished from cross-orientation inhibition through dichoptic measurements. Binocular cells which show end- or side-inhibition monopictically, inhibition can also be elicited dichoptically. In contrast, we were never able to elicit cross-orientation inhibition dichoptically.

A model is proposed to explain the spatial organization of end- and side-inhibition and cross-orientation inhibition in terms of cortical circuitry. (EY0175)


The question of knowing how far objects appear to be away from us is related to binocular distance perception which has to be distinguished from relative depth perception or stereopsis. Retinal horizontal disparity alone is not sufficient to allow either 1) the true distance of an object to be determined or 2) to make accurate judgments about the 3-dimensional shape of objects. This is because both types of judgments require additional information about the fixation distance which could originate mainly from the angle of vergence and/or the degree of accommodation and/or retinal vertical disparity. We have addressed the question of what happens to the response properties of individual neurons when the distance of fixation is changed. We recorded responses of neurons to static random dot stereograms in cortical area V1 of a monkey trained to perform a fixation task. We first studied 79 neurons for stereopsis at a fixation distance of 40 cm. About half of them (47%) were disparity sensitive. Thirty one neurons were also tested at more than one fixation distance (71%) than when only the 40 cm distance was used. By doubling the size of the image on the screen when the fixation distance of this screen was increased by a factor of two, it was possible to maintain the angular size of the stimuli constant. The luminance level was about 1 cd/m² at the 3 distance. In 22 cells, disparity sensitivity emerged only at certain viewing distances. Consequently, the proportion of disparity sensitive units was actually higher when the neurons were tested at more than one fixation distance (71%) than when only the 40 cm distance was used. For 9 of the 31 neurons, there were changes in the spontaneous activity of the neurons related to changes of viewing distances. In 6 of these cases, the spontaneous activity was increased when the animal was fixating at shorter distances. Our findings show that retinal horizontal disparity sensitivity of neurons as early as cortical area V1 is influenced by fixation distance and thus by extraretinal factors probably related to vergence.


Localizing of recording sites in behaving animals has been difficult because the visibility of electrode tracks and lesions declines rapidly with survival time. With current techniques which have well-localized damage (1.2 µm for 6 min), neither electrolytic lesions nor iron deposition clearly marked recording sites. In contrast, coating the electrode with the nonionic fluorescent carbocyanine dye, DiI, resulted in distinguishable tracks visible for at least 10 days.

During recording, electrode depth was monitored carefully. The cortical surface was visualized through a small slit in the dura and blood vessels were avoided to minimize damage. A biopsy was taken, fixed with glutaraldehyde/paraformaldehyde, and sectioned. Sections were photographed with a fluorescence microscope to map the electrode tracks. Then they were reacted for cytochrome oxidase to visualize the laminas, which obliterates the laminar boundaries. Laminar boundaries were drawn and the fluorescence photographs of the electrode tracks were projected onto the drawings to observe the trajectory of the electrode through the cortical layers. The depths determined in this way corresponded well to the electrode depths.

As previously reported (ARVO, 1988), cells in the superficial layers often had small receptive field activating regions with potent inhibitory surrounds. A powerful stimulus for these cells was a moving texture composed of small rectangles oriented to match the preferred orientation of the cell and configured to avoid simultaneous stimulation of the cell and the surround. Natural surfaces such as those of vegetation, earth, and animals often are imaged to produce such oriented textures. These patterns could be used by cortical neurons to contribute to the discrimination of objects from backgrounds. This suggests another perceptual role for upper layer neurons, in addition to the commonly mentioned ones of border extraction and spatial frequency analysis.


Optical imaging of the functional architecture of cortex, based on intrinsic signals, is an useful tool for the study of the development organization and function of the living mammalian brain.

Here we show that this technique is also suitable for exploring the brain of awake behaving primates. We designed a chronic sealed chamber which was mounted on the monkey skull over the primary visual cortex and permitted imaging experiments through a glass window. Restriction of head position alone was sufficient to minimize movement noise in the awake animal imaging experiments. High resolution imaging of the ocular dominance, and the blobs was achieved simply by taking pictures of the exposed cortex when the awake monkey was viewing video movies. Furthermore, the functional maps could be obtained without the noise reduction procedure of synchronizing the animal respiration and the data acquisition to the EKG. The wavelength dependency and time course of the intrinsic signals was similar in anesthetized and awake monkeys indicating that the signal sources were the same.

We therefore conclude that optical imaging is well suited for exploring functional organization related to higher cognitive brain functions of the primate as well as providing a diagnostic tool for delineating functional cortical borders and assessing proper functions of human patients during neurosurgery.

Supported by IBM and the Kilis Family Foundation.
405.1 HETEROGENEITY AND COMPARTMENTALIZATION IN THE OLFACTORY BULB OF ELASMOBRANCH FISHES. L. Drayer and P.P.C. Graziadei. Florida State University, Department of Biological Sciences, Tallahassee, FL 32306.

Anatomical observations reveal that the Elasmobranch olfactory bulb is an elongated structure immediately adjacent to the olfactory organ. Depending on the species, it appears as a succession of swellings (i.e. the Bonnethead shark, Sphyraena tiburo) or as two independent subunits (i.e. the Sharp-nosed shark, Rhizoprionodon.variantus). We examined several specimens in order to understand the functional significance of these morphological features.

The olfactory bulb of all specimens stained with Golgi method exhibit two distinguishable populations of mitral cells. One type has loose dendritic arborization bearing numerous terminal boutons. The second type of mitral cell, smaller, shows an extremely dense feather-like arborization.

Localised extracellular injections of Biocytin in the olfactory epithelium result in restricted labeling of the bulb, suggesting a topographical arrangement of the olfactory projections onto the bulb. The lateral olfactory bulb would process information coming from the lateral epithelium, whereas the medial bulb would process information coming from the medial epithelium. Several sizes of glomeruli were also observed with this method. Further experiments are in progress to delimit the projection fields with accuracy and define the lateral interconnections between the subdivisions of the olfactory bulb.


The mitral/tufted cells (MTCs) of the rat accessory olfactory bulb (AOB) are morphologically different from the mitral and tufted cells of the main olfactory bulb (MOB); the MTCs have more diverse dendritic branching and unique gliad-like microtubules (GMs). To identify the developmental sequence of the MTCs dendritic branching and the appearance of the GMs we have studied the MTCs in the rat AOB during the postnatal period.

Using the rapid Golgi-Golgi-Kopsch methods, immature MTCs were observed in the rat AOB from postnatal P1 to P14. The mitral and tufted cells of the MOB were also observed at comparable stages. At P1-P2, MTCs had dendrites branching in beaded structures, however, the GMs were not yet observed. GMs were often observed in the mitral and tufted cells of the MOB. At P8-P10 dendrites of the MTCs began to show branching and early formation of GMs. At P15, GMs and complex branching of the dendrites acquired more complexity; morphological details as observed in the adult were obvious by P22.

Our observations indicate that the MTCs and their specific intraglomerular GMs develop postnatally and at later stages than the GMs of the mitral and tufted cells of the MOB. Supported by a grant from the NIH (NS 20699) to P.P.C.G.

405.5 FLUORESCENT TRACT-TRACING STUDIES OF DESCENDING PROJECTIONS FROM THE INSULAR CORTEX TO THE NUCLEUS TRACTUS SOLITARIUS. T. S. Donta and J. A. London. Center for Neurological Sciences and Dept. of BioStructure and Function, Univ. CT Health Center, Farmington, CT 06030.

This laboratory has been investigating descending cortical projections to the taste-responsive rostral pole of the nucleus tractus solitarius (NTS) in golden Syrian hamsters (Mesocricetus auratus). Previous work in this lab has demonstrated that the insular projection is greater than the ipsilateral projection, and that the contralateral projection area extends further dorsally in layer 5 than the ipsilateral projection area. The rostral-pole of the NTS is primarily innervated by the chorda tympani nerve. To expand our investigations to include descending cortical projections to other regions of the NTS, injections of retrograde tracers into NTS regions were guided by multi-unit electrophysiology. Injections of WGA-HRP into the NTS were also observed at comparable stages. At P1/P2 MTCs were observed in the rat AOB from postnatal P1 to P14. The mitral and tufted cells of the MOB were also observed at comparable stages. At P1-P2, MTCs had dendrites branching in beaded structures, however, GMs were not yet observed. GMs were often observed in the mitral and tufted cells of the MOB. At P8-P10 dendrites of the MTCs began to show branching and early formation of GMs. At P15, GMs and complex branching of the dendrites acquired more complexity; morphological details as observed in the adult were obvious by P22.

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The monkey muscle Parkin Cell Degeneration (PCD) loses all mitral cells (MCs) in the olfactory bulb (OB) over a period of about 30 days beginning at 4 mon. of age. Tafted cells and denervated granule cells subsequently exhibit significant local synaptic reorganization (5). The present study investigates the development of the neonatal vomeronasal (VN) system. Neonates survived an additional 1, 7, 14, 21 or 28 days following injection and most prominently in the lateral PBN. The SC showed a marked reduction in the number of cells to incorporate c-H-thymidine after Day 14. For 1 day survival groups, injections of c-H-thymidine in one or 3 radioactively labeled stem cells through the primitive VN organ while injection made after Day 7 selected labeled stem cells in the developing VN organ at the basal cell layer. For 21 day survival, c-H-thymidine labeled stem cells in the intermediate zone of the sensory epithelium where they sustained a life span exceeding 28 days. Furthermore, tenecephalic neurons generated prior to Day 3 first differentiated into mitral cells in the main olfactory bulbs (MOB) by Day 8 and in the accessory olfactory bulbs (AOB) by Day 15. Injection made on Day 7 and thereafter labeled neurons which gave rise to the granule cells predominantly and very few mitral cells in the MOB and AOB. Supported by NIH RR-4570, NS-11713 and MUSOM.


The connectivity of the area postrema (AP) was investigated in the hamster. Intraperitoneal injections of WGA-HRP were made into either the central portion of the AP or the parabrachial nucleus (PBN). After 24-48 hours survival time, animals were perfused with 0.5% glutaraldehyde and 0.5% paraformaldehyde followed by a diffusion saline solution in phosphate buffer. Brains were removed in toto and processed for HRP histochemistry with tetramethylbenzidine. Alternate sections were mounted unstained with the remaining section counterstained with a Nissl stain.

PBN injections resulted in the labeling of numerous neuronal perikarya throughout the AP, medial and commissural subdivisions of the solitary complex. Injections of the lateral PBN were most effective in labeling of AP neurons. Prominent labeling was present in the AP but not prominent. Prominent labeling of the AP injections was present in the solitary complex at the level of the injection and most prominently in the lateral PBN. The area postrema of the hamster is in a pivotal position to receive not only primary afferent information but also information from the systemic blood due to a weak blood-brain barrier. The AP may play an important role in modulating visceral function by influencing pontine visceral relay centers. 


Five groups of neonatal South American opossums at the ages of 1, 3, 7, 14 and 28 days (N=44 total) were injected with a single dose of H-thymidine (2 to 5 µCi, i.p.) for tracing the development of their vomeronasal (VN) system. Neonates survived an additional 1, 7, 14, 21 or 28 days following injection and were processed into light microscopic autoradiographs on slides.

For 1 day survival groups, injection made after Day 7 selectively labeled stem cells in the developing VN organ at the basal cell layer. The second type of mitral cell, smaller, shows an early period of development, but loses its axon and never contributes to the sensory epithelium where it sustained a life span exceeding 28 days. Furthermore, tenecephalic neurons generated prior to Day 3 first differentiated into mitral cells in the main olfactory bulbs (MOB) by Day 8 and in the accessory olfactory bulbs (AOB) by Day 15. Injection made on Day 7 and thereafter labeled neurons which gave rise to the granule cells predominantly and very few mitral cells in the MOB and AOB. Supported by NIH RR-4570, NS-11713 and MUSOM.


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Our observations indicate that the MTCs and their specific intraglomerular GMs develop postnatally and at later stages than the GMs of the mitral and tufted cells of the MOB. Supported by a grant from the NIH (NS 20699) to P.P.C.G.
PARALLEL PROCESSING OF CHEMOSENSORY INPUT IN THE BRAIN OF THE SPINY LOBSTER. McSchimm, F. Orsino and D.W. Ach. Whitney Lab. of Neurosciences and Neurology, Univ. of Florida, St. Augustine, FL 32086

The biramous antennules (1st antennae) of decapod crustaceans are complex sensory organs that integrate olfactory (acetabular), mechanosensory, and chemosensory stimuli. To analyze the central processing of chemosensory information we backfilled antennular afferents and intracellularly recorded and stained dendritic arborizations in the spiny lobster antennuloneuromotor center. The antennuloneuromotor center contains two distinct pathways for processing antennular input. One pathway includes the olfactory, parolfactory, and accessory lobes (OL, PL, AL), and the second pathway includes the lateral and submedian antennular neuropits (LAN, MAN), which lack glomerular organization and receive olfactory- and non-olfactory chemosensory input from both flagella and the basal segment of the antennule. A major output component of the LAN and MAN are antennular motoneurons whose neurites are interweaved with the afferent projections. The second pathway appears to mediate local antennular reflexes (LAN) and coordinate reflexes of the head appendages to antennular stimulation (MAN). The independent processing of olfactory and non-olfactory chemosensory input originating from the same antennule further argues that olfaction is a distinct sense in invertebrates, aquatic animals.

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The cone electrode (mainly composed of an insulated gold wire) was implanted into the tip of the cone electrode and remained viable for 15 months as shown by EM studies and by histochemical staining. After removal of the electrode for as long as 15 months, the cone electrode was reconstructed with camera lucida. The terminal arbor of single axons encompassed areas of 41-1840 μm² (mean ± SEM = 6.84 ± 1.44). The number of axon bifurcations varied from 2 - 19 (mean ± SEM = 5.91 ± 0.51). Further studies revealed that a single terminal enlargement or varicosity could make synaptic contact with more than 1 postsynaptic target. These data show that olfactory receptor cell axons segregate into small subglomerular compartments. This differential innervation could provide a basis for subglomerular organization and perhaps differential synaptogenic organization.

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To establish structure-function relationships in the NST, and as a prelude to future developmental studies, we have begun an investigation of the morphology of taste-sensitive neurons in the adult rat brainstem. Gliss micropipettes filled with 6.0% HRP in Tris-KCl buffer were advanced into the rostral NST and a bipolar stimulating electrode was placed on the ipsilateral chorda tympani (CT) nerve. The response properties of neurons driven by the CT stimulating electrode were tested using 0.5 and 1.0 M NaCl, 0.5 and 1.0 M KCl, 0.01 M quinine HCl, 0.01 M NaCl and 1.0 M sucrose. Following receptive field characterization the cells were implanted and injected with HRP. Cells were grouped according to soma size and shape, axonal target and dendritic field morphology. Most of the neurons recovered in this initial phase of our study have been quite small, with soma diameters in the range of 10-15 μm, and have responded best to NaCl or quinine HCl. The majority of these neurons do not project to rostral targets, but instead appear to terminate in the NST or adjacent regions of the brainstem. Our results suggest that the intracellular labeling technique will permit us to relate the physiological and morphological properties of gustatory neurons in the NST.

CORTEX: ANATOMY


The cone electrode serves as the foundation for a long-term recording (LTR) of neural signals or potential clinical and investigative applications. To facilitate LTR a novel cone electrode was inserted into a hollow glass cone within a collar of brain tissue and embedded in agar. The glass cone was fixed during recording and postfixed in osmium tetroxide. The subcortical layers of the brain were cut into 40 μm sections and reacted for marker proteins, blood vessels, and glial profiles. Neural axons and dendrites were selectively labeled with HRP, horseradish peroxidase, and the transport marker HRP, horseradish peroxidase. The stained neuronal elements were stained for electron microscopy and projections were observed in the cortex. The cone electrode was used to label anterior cortical regions and to label posterior cortical regions. The cone electrode was used to label anterior cortical regions and to label posterior cortical regions.


A monoclonal antibody to a neurofilamentous protein (SMI-32, Sternberger Monoclonal Inc.) labels a subpopulation of pyramidal neurons in the cortex. This study was focused on the motor and premotor areas of the brain. The distribution of labeled neurons varied substantially across cortical areas (Kennedy et al., 1989) and preparations indicated connections between neurites within the cortical columns and surrounding cortical neurons. The electron microscopic study now further defines the structure and function of the following implantsation for as long as 15 months. After removal of the electrode, the tissue was fixed and stained for electron microscopy.

These studies suggest that cortical neurites grow into the tissue of the cone electrode and remain viable for 15 months as shown by EM studies and by recordings of movement-related activity in the same monkey. A preponderance of neurofilamentous protein in the above frontal areas may be related to the specialization of the cortical motor areas. (Supported by grants NS28571, NS24760, and BRSG955-SAR)
406.4 **A COMPARISON OF FRONTAL LOBE INPUTS TO THE PRIMARY, SUPPLEMENTARY AND CINGULATE MOTOR AREAS IN THE MONKEY.** R.J. Morecraft and G.W. Van Hoesen, Dept. of Anatomy and Neurology, Univ. of Iowa, Iowa City, Iowa 52242

Although frontal lobe interconnections of the primary (area 4 or M1) and supplementary (medial area 6 or M2) motor areas are well understood, how frontal association cortex influences these or other areas that give rise to the corticospinal tract is not. We investigated the distribution of frontal lobe inputs to the primary motor area (areas 24c and 23c) in the rhesus monkey using fluorescent dyes. M1 received input from area 6, M2 and cingulate areas 24c and 23c. M2 received input from all corticofugal types of cortex within this area including areas 10, 46, 9, 12, 8B, 8A, 6, M2, M1 and subfrontal areas. A double labeling experiment demonstrated that less than 1% of all labeled neurons in areas 46, 9, 6, 4C and M2 send axon collaterals to areas 24c and 23c. The heavy component of cortoradial frontal input to M3 suggests a hierarchy concerning the diversity of frontal lobe inputs. M1 receives the least diverse frontal lobe input which arises exclusively from primary association cortex. M2 receives more diverse input which arises from multimodal association, primary association and primary motor cortices and M3 receives the most diverse and widespread frontal lobe input which includes paralimbic, multimodal association, primary association and primary motor cortices. These patterns of connectivity suggest that frontal association input gains preferential access to the cortical motor areas via M3. (Supported by grant NS14944)


An ultimate goal of the use of fluorometric techniques with 14C-deoxyglucose autoradiography is to define a unilaterally 6-hydroxydopamine lesion of the substantia nigra. Animals were studied both at rest and with a sensory challenge. Primary motor, sensory and secondary motor regions all showed alterations in local cerebral glucose utilization (LCGU) associated with the lesion, but not more than 15%. Lesion animals showed side-to-side significant decreases in glucose utilization not shown in sham lesion controls, with a decrease in LCGU ipsilateral to the lesion. Mean cortical LCGU of all 73 regions analyzed was significantly decreased relative to untreated controls bilaterally (4%, p<0.01). The response of the cortex to the sensory challenge was also modified by the lesion. The results demonstrate a small but significant and widespread change in neural function related to dopamine depletion of the basal ganglia.

406.6 **PREFRONTAL, PREMOTOR, AND HIPPOCAMPAL CONNECTIONS OF AREA 24 IN THE MACAQUE MONKEY.** T. Arikuni, H. Sakai*, and A. Murata*, Dept. of Anatom. and Neurophysiol., Nihon Univ. Sch. of Med., Tokyo 173, Japan

The present study was undertaken to clarify the organization of transcallosal connections between the dorsolateral prefrontal cortex and the hippocampal formation in the macaque monkey. Under direct vision, small amount of 10% solution of wheat germin conjugated horse-radish peroxidase (WGA-HRP) was injected into each of several regions of area 24 of the cingulate cortex in the Macacafuscata. The connections of prefrontal cortex, premotor area, motor area and the temporal cortex connected to the hippocampus were analysed. WGA-HRP labeled cells and terminals appeared densely in areas 10, 9 and 13 and in the rostral area 46, while labeled cells and terminals appeared patchily in the caudal area 46 and areas 8A and 45. HRP-labeled cells and terminals were distributed more extensively in area 6a than in areas 6ax and 6b. Labeling was seen in areas 4a and 4b of the motor cortex and in the prosomucium of the hippocampal formation. HRP-labeled cells were distributed in layer V of areas 28 (the entorhinal area) and Th, while they were distributed in layers III and V of areas 35, 36, TF, and TE. All of these areas contained HRP-labeled terminals. In summary, the prefrontal cortex and prefrontal area have strong reciprocal connections with area 24 which is strongly reciprocated with the prosomucium and parahippocampal area.


To elucidate subcortical inputs to the supplementary motor area (SMA) precisely, we labeled pallidal or cerebellar terminals in the thalamus using wheat germ agglutinated horseradish peroxidase (WGA-HRP), and labeled thalamic neurons projecting to the SMA using Fast Blue (FB) in the same monkey. FB was injected into the rostral area of the SMA after confirming the somatotopy using intracortical microstimulation. After 3-4 weeks, WGA-HRP was injected into the internal segment of the globus pallidus or the deep cerebellar nuclei. After 3 days, the animals were perfused under deep anesthesia. The sections were processed with tetramethylbenzidine and observed with bright-field and epifluorescent illumination. Numerous FB labeled thalamic neurons were distributed in pallidal terminal areas. In contrast, few labeled neurons were found in cerebellar terminal areas.

The present results indicate that the SMA receives more cortical inputs from the basal ganglia via the thalamus.

We used injections of two different fluorescent tracers to demonstrate that primary motor cortex (MI) and supplementary motor area (SMA) receive some inputs from separate regions of cortex, and some from the same regions, although few neurons project to both fields. Forelimb and hand regions were first identified by intracortical microstimulation, boundaries were marked with microlesions and different tracers were injected into the same body part representation in both fields. Brains were sectioned in the plane or parallel to the surface after flattening, and alternate series of sections were stained for myelin, CO or AchE. Injections revealed arrays of short intrinsic connections in both fields and interconnections between the fields. MI had major inputs from areas 3a and 1, while SMA did not; neither area received notable input from area 3b. More caudally cortex in the 2–5 region projected to both SMA and MI, although only a few double labeled neurons were detected. Almost all the projections from SII region and adjoining cortex were to MI. Foci of labeled neurons projecting to MI and SMA from lateral premotor and rostrally adjacent cortex overlapped extensively, but few cells were double labeled. Some regions of cingulate cortex projected to both fields, but rarely via the same neurons. Finally, cortex on the medial wall just rostral to the SMA and more caudal cingulate region sent projections to SMA but not MI. (Supported by NS 16446).

406.10 AREA LIP INPUT TO AREA 7A IN THE INFERIOR PARIETAL LOBULE OF MACAQUE. J.R. Tian. J.C. Lynch, and S.P. Hardy, Department of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216.

Studies of the sources of neural input to area 7a, a higher-order association area in the inferior parietal lobe, have been inconclusive about whether there is a significant projection to 7a from the lateral intraparietal area (LIP), a region in the lateral bank of the intraparietal sulcus that seems to have predominantly visual and oculomotor functions. This question was addressed by making small placements of the retrogradely transported tracers diamidino yellow or fast blue in area 7a and/or LIP in 3 M. fascicularis and 1 M. mulatta. Labeled neurons were counted in every 2–3 sections through the LIP, cingulate gyrus, and the principal sulcus region of prefrontal cortex (PS). Typically, both cingulate gyrus and PS cortex contained more than twice as many neurons that projected to 7a as did LIP. Neurons in LIP that were labeled by 7a injections were restricted to a single small zone. In contrast, neurons in 7a that were labeled by LIP injections were distributed widely through the area. More neurons were labeled in 7a by the LIP placements than vice versa. These experiments confirm that 7a is a source of neural input to 7a, but suggest that, in spite of the close physical proximity of 7a and LIP, and of their functional similarities, both the cingulate gyrus and the PS region of prefrontal cortex provide more robust neural connections with 7a than does LIP. Furthermore, the data suggest that the projection from 7a to LIP may be considerably more extensive than the projection in the opposite direction. (Supported by PHS grant EY04159)

406.11 PATTERN OF PROJECTIONS FROM THE PREMOTOR AREAS ON THE MEDIAL WALL OF THE HEMISPHERE TO THE PRIMARY MOTOR CORTEX (MI) AND SUPPLEMENTARY MOTOR AREAS (SMA) IN MACAQUE, M. fascicularis AND M. mulatta. A. Guldin, S. Morling* and O.-J. Grinner, Dept. of Physiology, Free University Berlin, 1000 Berlin 33, Germany.

The representation of the distal forelimb in the primary motor cortex of macaques is found in 2 regions: 1) a rostral region on the crest of the precentral gyrus and 2) a caudal region in the anterior bank of the central sulcus. The results of prior studies have suggested that anatomical and physiological differences exist between the 2 regions of distal forelimb representation (e.g., Strick and Preston, ’82; Holsapple et al., ’91). We used retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) to examine the pattern of connections between the arm areas of the premotor areas on the medial wall of the hemisphere and the rostral and caudal regions of forelimb representation in the primary motor cortex (MI) and supplementary motor area (SMA). Tracer injections into the lateral ventral nucleus (VPL, VPI) or the rostral and caudal regions of the supplementary motor area (SMA, n=2) and the caudal cingulate motor area on the ventral bank of the cingulate sulcus (CMAd, n=2) stimulated large numbers of neurons in either region of forelimb representation on the crest of the precentral gyrus. Surprisingly, few neurons were labeled in the caudal region of the forelimb representation in the anterior bank of the central sulcus. In contrast, tracer injections into the caudal cingulate motor area on the dorsal bank of the cingulate sulcus (CMAd, n=2) stimulated large numbers of neurons in the caudal region of the central sulcus, as well as in the rostral region. These results support the concept that the rostral and caudal portions of the forelimb representation in the primary motor cortex each have a unique input-output organization. Furthermore, they provide additional evidence that the CMAd is a motor field which is distinct from the SMA and CMAv. Support: VA Med.Res.Serv.; USPHS 2957, 24328, 843902.


The retroinsular cortex (RI) is a cortical representation of the somatosensory and motor areas, situated in the posterior Sylvian sulcus of the marmoset monkey (Callithrix jacchus). Different retrograde tracers were placed into the cortical regions around this sulcus. The loci of injection were identified by single unit recordings and subsequent histology.

Thalamic aff erences originate mainly in the oral- and mediial-pulvinar nucleus (PUO, PUM) in the posterior group PG and in the lateral ventral and the inferior ventral nucleus (VPL, VPI). The cortical input to RI of the Callithrix monkey comes from the area 7, the cingulate cortex, the somatosensory areas SI and SII and the insula. Our results support in part our former findings on the interconnections of the parieto-insular vestibular cortex (PIVC) of the Squirrel monkeys.

406.13 EFFERENCES FROM THE VESTIBULAR CORTEX TO THE VESTIBULAR NUCLEI IN PRIMATES. E. Akhabian, O.-J. Grinner and W. Guldin, Dept. of Physiology, Freie Universitat Berlin, 1000 Berlin 33, Germany.

The parieto-insular vestibular cortex (PIVC) is a vestibular cortical field located in the posterior insula and the retroinsular cortex. Vestibular neurons were found in the PIVC of Java monkey (Macaca fascicularis) and Squirrel monkey (Samirius sciureus). Identifying injection sites by recordings and unit responses, we placed different retrograde tracers in the vestibular nuclei (VN) of anesthetized Java and Squirrel monkeys. In both species retrograde nerve cell labelling in the PIVC region could be detected. The only other cortical regions with noteworthy labelling after VN injections were area 7 and the cingulate gyrus. These findings indicate that the PIVC is the main cortical region of vestibular signal processing.

406.14 VIBRISSEL MOTOR CORTEX IN THE ADULT MOUSE; ITS CORTICAL AND THALAMIC INPUT.

Ulrich, N.*, E. Welker* and H. Van der Loos, Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

In order to study the potential role of a somatosensory pathway in adult plasticity in a related motor pathway we have investigated their interrelationship in the vibrissal system (PIVC) and also the posterior complex of the thalamus (PO). We delineated a discrete portion of the primary motor cortex where, under light anesthesia, movements of individual vibrissae could be elicited. This cortical locus we designated the fluorescent retrograde tracer (iodamine beads), while an anterograde tracer (biocytin) was injected in the barrel in SI corresponding to the stimulated area (as determined physiologically, in the same animal, n=10). The vibrissal motor cortex receives input from the barrel cortex and the following thalamic nuclei: ventral lateral (VL), ventral medial (VM), medial ventral posterior complex (PO), zona incerta (ZI) and some of the medial thalamic nuclei (MT). Future analysis will further define the significance of PO as a link between the somatosensory vibrissal pathways. Support: Swiss NSF 3100.009468.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
406.15 INTERHEMISPHERIC COMPARISONS OF THE ORGANIZATION OF BRODMANN'S AREAS 44 AND 45 IN HUMAN BRAIN. T.L. Hayes and D.A. Dennis. Division of Experimental Neurosciences and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

Although motor speech functions are believed to be localized to the left hemisphere, information about how the cortico- and chemoarchitecture of these areas compare to their homologues in the right hemisphere is scarce. We used Nissl stains to compare the cytoarchitectonic features of these regions in five control human brains. Areas 44 and 45, located in the posterior inferior frontal gyrus, were identified according to published criteria. At its most caudal extent, area 44 is roughly similar. More rostrally, in area 45, the cortex becomes granular, with unusually large pyramidal neurons present in deep layer III. At the most rostral aspect of area 45, the large layer III pyramidal neurons were not seen. These cytoarchitectonic features were identified in both hemispheres. However, measurements of the size of the large layer III pyramidal neurons in caudal area 45 revealed that they were larger in the left hemisphere than in the right (p < .05); the largest neurons were 10-20% larger in the left than in the right. Further comparisons were made using immunohistochemical markers of non-phosphorylated neurofilament protein (NPWF) and calbindin. As previously reported (Neuro. Abstr. 16:240, 1990), the large layer III pyramidal neurons in the left hemisphere were immunoreactive for NPWF but not for calbindin. The present study revealed a similar pattern of immunoreactivity in the right hemisphere. Further investigation of possible hemispheric differences in these regions is underway. These studies may provide insight into the anatomical basis of the lateralization of motor speech function.


Previous analyses combining retrograde transport of Fast Blue (FB) with immunohistochemistry have demonstrated that a substantial sub-population of cortico-cortically-projecting neurons (CCP) contain high concentrations of non-phosphorylated neurofilament protein (NPWF). However, the relative proportion of a given projection that is NPWF+ varies with its functional role and connectivity characteristics (Campbell et al, Brain Res 91, 1991:359). For these analyses, FB injections were placed in one of the following regions: prefrontal cortex ventral to the principal sulcus (VIPS), inferior parietal cortex (vIPS) or cortex in the superior temporal sulcus (STS). The distributions of FB only and FB plus NPWF double labeled neurons were analyzed in regions that provide cortical projections to each injection site. Quantitative analyses revealed that: 1) the intracortical connections between these three association areas vary in the proportion of CCP that are NPWF+ such that connections between vIPS and STS have the lowest proportion (30-40%), vIPS and vIPS intermediate (40-50%), and STS and vIPS the highest (75-90%). 2) Reciprocal connections tend to have a similar proportion of double labeled cells. 3) The incidence of NPWF+ CCP from homologous contralateral areas varies from 25-100% depending on the system involved. 4) Analyses within the visual system suggest that CCP within a given sensory modality may display differential topological characteristics as compared to CCP interconnecting high level association regions. Supported by AHAF and NIH AG06647.


The cells of origin of projections from the ventromedial, ventralis, and interstitial thalamic complexes, mfnluc, and visceral motor, visceral-sensory, and an important role in the orbito-insula and most of the insula proper but also to the orbito-insula and most of the insula proper. However, the posterior association areas generally project to the orbito-insula and most of the insula proper. Moreover, a standard oculomotor delayed response task (VODR) and a spatial delayed response task (SODR) to the memory-guided saccades directed toward the target contralateral to the delay. Further, the effective sites of the injection were clustered in a small region of the dorsal bank of principal sulcus. The data suggest that the region can be considered a working memory center for visuomotor and somatosensory working memory for spatially guided behavior.


To examine the modality-specificity of spatial working memory mediated by the prefrontal cortex (PFC), bicuculline was injected into the dorsolateral PFC of monkeys that were trained on 3 different oculomotor tasks associated with memory-guided saccades to left or right spatial targets: 1) a standard oculomotor delayed response task (VODR); 2) the oDK task by auditory stimuli (AD); 3) a delayed alternation task where the monkeys' previous saccade responses presumably guided the monkey, or the monkey's previous visual input. In each daily session, the monkeys performed the VODR and one or two of the other memory tasks as well as a control task which required the monkeys to make only sensory-guided saccades. Several delays (1-5 sec) were employed in each task. Local injection of bicuculline (1-8 μg) into the PFC induced deficits in all of the memory tasks but not in the control task. When the VODR task was impaired, the AODR and SODR tasks were invariably impaired, and vice versa. The deficits were usually restricted to the memory-guided saccades directed toward the target contralateral to the injection site, and the impairment was dependent on the duration of the delay. Further, the effective sites of the injection were clustered in a small region of the dorsal bank of principal sulcus. The data suggest that the same region of the dorsolateral PFC is involved in visual, auditory, and somatosensory working memory for spatially guided behavior, and that this region can be considered a working memory center for spatial cognition.

407.3 CORTEX: LESION AND STIMULATION

CORTEX: LESION AND STIMULATION

WEDNESDAY PM CORTEX: ANATOMY 1021

407.4 INFLUENCE OF THE CAT ON THE REPRODUCTION TRANSPORT OF HOMERIZED INJECTION IN THE VENTRAL GROUP, MEDULLA AND NUCLEUS VISCEROSOMATIQUE AND NUCLEUS VISCERALIS OF THE SCALE

407.5 INSULAR CORTEX OF THE CAT: THE RETROGRADE TRANSPORT OF HOMERIZED PERIODOTOXIC TO THE VENTRAL GROUP, MEDULLA AND NUCLEUS VISCEROSOMATIQUE AND NUCLEUS VISCERALIS OF THE SCALE

407.6 CORTEX: LESION AND STIMULATION

407.7 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

407.8 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

407.9 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

407.10 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

407.11 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

407.12 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

407.13 CORTICAL DAMAGE OR ACUTE INJECTION: EFFECTS ON THE SPATIAL WORKING MEMORY. D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.
407.3 

We carried out extensive motor mapping studies of the dorsolateral frontal lobe in seven owl monkeys (Aotus trivirgatus). Animals were anesthetized with ketamine and xylazine and stimulated intraorally with 5—1 MEG microelectrodes. Under these conditions, somatic and ocular movements could be elicited from a large territory to primary motor cortex (M1); only the extreme ventrolateral and polar regions were unresponsive at high currents. A dorsal premotor region lies between the low-threshold (<40 μA) M1 and the region that is agranular in architecture, like M1, but has smaller pyramidal cells; somatic movements could be elicited at currents of ~40—150 μA, and sometimes less. An additional somatosensory region is located just rostral to the M1 face representation. Forelimb (and more laterally) face movements were elicited from this zone, often with low currents (<40 μA). This zone is dysgranular, rather than agranular, and is probably homologous to the arcuate premotor area (APA) of macaques. Rostral to the premotor zone, eye movements were elicited at low thresholds from the frontal eye field (FEF) and supplementary eye field (SEF), and at higher currents from the cortex located just rostral to FEF (previously described as the frontal ventral area, FVA). Evoked potentials in FEF and SEF are also reported.

In the latter region, thresholds typically ranged from ~50—200 μA, and at many points, movements of the face as well as the eyes could be elicited. (Supported by NS 16446, EY 06304, and JSMF #90-35.)

407.5 
DYNAMIC MODULATION OF PRIMATE MOTOR CORTEX OUTPUT DURING MOVEMENT, L. P. Donoghue and J. N. Nisen, Center for Neurobiology and Human Behavior, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15213.

These experiments tested the hypothesis that motor cortex output is dynamically modulated during skilled arm movements. M. fasciculata monkeys performed a visually guided reach task which required flexion of the wrist against a spring load. During movement preparation and execution the motor cortex was stimulated at one of 11 sites through 70 μm Pt-Ir wires that were chronically implanted in areas 4 and 6. The EMG was recorded in 7 arm muscles (biceps, triceps, ECU, ECR, PL, EDC, FCU). Individual sites were stimulated with 30 ms pulse trains below 20 μA in each of 6 phases: (1) hold (no load), (2) during the same hold but after target location was displayed, (3) 250 ms after a go cue, (4) during movement initiation, (5) at mid movement, and (6) during hold in target zone. Modulation and/or augmentation of evoked EMGs was site dependent. Stimulation at sites where digit movements were elicited at threshold showed only suppression or no change in EMG evoked from digit or wrist extensors. Pronounced modulation of FCU, ECU, ECR, or PL, (1-5 fold increase of evoked EMG above 20 μA) was seen at wrist and shoulder or mixed movement sites, most frequently in epoch 3 (68% of cases). Evoked EMG during epochs 4-6 at these sites were typically equal to or below that evoked in epoch 1, even though digits were moved around. All sites evoked EMG in wrist muscles paralleled expected changes in the excitability of wrist flexor motor neuron pools. Modulation due to the instruction was uncommon (19% of the muscle observations). The occurrence of evoked EMG could depend on the stimulation epoch occurred: 39% of sites a muscle was activated from cortex only in 3 epochs. These findings suggest that the relationships between groups of cortical neurons and muscles may be temporarily maintained during different movement epochs. This organization may direct feedback onto selected cortical cell groups and could be used to coordinate different body parts with a single movement.

407.6 
MUSCLE INHIBITION FROM TRANSCRANIAL STIMULATION IN NORMAL HUMANS, E. M. Wasserman, A. Paciulli-Leone, J. Vali-Sole, L. G. Cohen, and J. Halsted, Human Cognitive Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

We used transcranial magnetic stimulation to study 5 normal subjects for inhibitory motor responses. Subjects activated the abductor pollicis brevis (APB) while we stimulated sites one cm apart on the contralateral scalp with a Cadwell MES-10 stimulator and an "S" shaped coil. Recalled EMG from APB was averaged in area where stimulation produced the largest motor evoked potentials (MEPs), low stimulus intensities (30-35% stimulator output) elicited silent periods (SPs) on 20 trial averages without evidence of preceding facilitation. At M30 SPs were elicited. Higher intensity (M50) MEPs followed by SPs were found at a cluster of sites on the contralateral scalp (MEP area). At the same stimulation intensity, averages from 3 to 9 sites just anterolateral to the MEP area yielded SPs without facilitation. SPs without facilitation could not be produced at any other sites at this intensity. Areas just lateral, high intensities (100-100%) evoked robust SPs without MEPs on single trials. Muscle suppression without preceding facilitation may be mediated by low threshold corticospinal neurons, spinal circuits excitable by weak cortical output, or inhibitory cerebral circuits. The difference between MEP and SP thresholds is greatest anterolateral to the center of the MEP area. This region may correspond to an inhibitory motor area defined by direct cortical stimulation with electrical pulse trains.

407.7 
CORTICOSPINAL PROJECTIONS TO LOWER LIMB MUSCLES IN MAN. B. Brouwer and P. Ashley, Dept. of Rehab. Therapy, Queen's University, Kingston, ON K7L 3N6 and Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, ON M5T 2S8.

The relative strength of the projections from cortical neurons activated by transcranial magnetic stimulation to lower limb spinal motoneurons of the vastus lateralis and the ocular cortex. This region is agranular in architecture, like M1, but has smaller pyramidal cells; somatic movements could be elicited at currents of ~40—150 μA, and sometimes less. An additional somatosensory region is located just rostral to the M1 face representation. Forelimb (and more laterally) face movements were elicited from this zone, often with low currents (<40 μA). This zone is dysgranular, rather than agranular, and is probably homologous to the arcuate premotor area (APA) of macaques. Rostral to the premotor zone, eye movements were elicited at low thresholds from the frontal eye field (FEF) and supplementary eye field (SEF), and at higher currents from the cortex located just rostral to FEF (previously described as the frontal ventral area, FVA). Evoked potentials in FEF and SEF are also reported.

In the latter region, thresholds typically ranged from ~50—200 μA, and at many points, movements of the face as well as the eyes could be elicited. (Supported by NS 16446, EY 06304, and JSMF #90-35.)

407.8 
ACTIVATION OF LOWER LIMB MUSCLES BY MAGNETIC STIMULATION IN SPINAL CORD INJURED (SCI) PATIENTS: A CONTEXT DEPENDENT STUDY. L. C. Vergeer, B. E. Mustard, and C. D. MacKenzie, Departments of Rehabilitation Medicine and Physiology, University of Toronto, Lyndhurst Hospital, Toronto, CANADA M3T 1WG.

Magnetic stimulation (MS) to motor cortex activates contralateral lower limb compound motor action potentials (CMAPs) in normals during different motor tasks. MS in SCI has been used to determine integrity of the corticospinal pathway but not as an element of neural recovery or therapeutic intervention clinically. SCI subjects with incomplete lesions demonstrate an ability to cycle with progressively more phasic and reciprocal activity during the recovery phase. We used MS to activate lower limb CMAPs (biceps femoris BF, rectus femoris RF, tibialis anterior TA, and soleus S) in these patients at rest, during selective muscle activation and during upright cycling to determine whether MS has utility as a therapeutic strategy. In studies using MS to the motor cortex (4 cm posterior; 2 cm lateral to Cz; with a Cadwell MES-10 with a 9.5 cm coil) CMAPs were repeatedly elicited in Q and S during selective muscle activation but not routinely in BF and TA (n=1). Response latencies for Q and S were 23-30 ms, the 31ms respectively. In normals (n=10) BF, Q and TA were reliably elicited in all subjects and S in 80% of subjects with facilitation. In contrast to normal subjects, CMAPs in SCI subjects were not elicited during active cycling. A single MS stimulus interrupted cyclic S activity and elicited maintained S activity that lasted throughout the trial. The study suggests that the response to MS in SCI patients may be context dependent and indicative of existing spinal motor circuitry. Supported by Rick Hansen Man in Motion Legacy Fund #90-25.
407.9 INHIBITORY INTERACTIONS BETWEEN TRANSCRANIAL BRAIN STIMULI APPLIED OVER THE MOTOR CORTEX IN MAN. T. Kajarić*, M. Slevogt1, J. P. Charvet1, S. Matsumoto, I. Soon1, D. Thomeer and C. L. Marsden*. 1MRC Human Movement & Balance Unit, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

With ethical committee approval, we gave pairs of transcranial brain stimuli separated by intervals of 1-20ms over the motor cortex of 10 normal volunteers. Both electrical (Digitimer D180) and magnetic (Magstim 200 connected to a figure 8 coil) stimuli were used. EMG responses were recorded from surface electrodes over the contralateral first dorsal interosseus (FDI) muscle. In active muscle, responses to electrical stimulation at (1-1.2 ms threshold) were facilitated by a preceding conditioning stimulus (either electric or magnetic) whereas responses to magnetic stimulation were inhibited. This suggests that the inhibition of magnetically-evoked responses occurred at the level of the motor cortex. The amount of inhibition was dependent upon the latency of the conditioning stimulus. At high intensities of conditioning shock, the amount of inhibition was reduced, and in some subjects became a facilitation. The position of the conditioning stimulus was important. Moving the conditioning shock away from the motor strip decreased the amount of inhibition. However, conditioning stimulation of the hand area of cortex was not necessary to produce inhibition of responses evoked in the FDI. For example, conditioning stimuli applied at the vertex at an intensity subthreshold for producing EMG responses in the active FDI could still inhibit test responses evoked in the same muscle by stimulation over the hand area. The inhibitory interaction between pairs of stimuli is probably produced by a combination of several mechanisms, including refractoriness of pyramidal tract cells discharged by the conditioning stimulus, recurrent inhibition from pyramidal tract collaterals, and cortico-cortical inhibitory processes.

408.1 INVESTIGATION OF MOTONEURONAL AND PREMOTONEURONAL MECHANISMS OF PUDENDAL REFLEX MODULATION DURING MICTURITION IN THE CAT. B. Fedirchuk, L. Song* and S. J. Shefchyk. Depts. of Physiology and Anatomy, University of Alberta, Edmonton, AB, Canada.

This research was supported by the Medical Research Council of Canada.


A glycinergic innervation of the trigeminal motor nucleus is suggested from physiological studies which investigated the innervation of motor neurone pools following peripheral and cortical stimulation. Following stimulation of the inferior alveolar and masseter nerves an early and late component of the masseter motoneuron burst is observed. The early hyperpolarization is blocked by a systemic application of strychnine suggesting that the hyperpolarization is due to glycine (Nakamura, Y. et al. Brain Res. 579, 1993). Following repetitive stimulation of the masticatory cortex, rhythmic jaw movements are observed. A long period of the rhythmic hyperpolarization seen in the masseter motoneuron burst is blocked by strychnine. Both electrical (Digitimer D180) and magnetic (Magstim 200 connected to a figure 8 coil) stimulation was used to elicit the trigeminal motor nucleus of male albino guinea pigs. Following a 72 hour survival time, the animals were trancanillicly perfused with 4% glutaraldehyde and 0.5% paraformaldehyde. Glycinergic innervation was performed using a polyclonal antibody (Chemicon, Inc.). ABC histochemistry with DAB as a chromagen was used to label the immunoreactive complex. Neurons which co-localized the connectivity marker and neurotransmitter marker were identified bilaterally in the paraventricular reticular formation, supragingivical nucleus, spinal trigeminal nucleus oralis and lateral paranaragangiomatous. Immunoreactive cells, not containing the retrograde marker, were identified in the superior olivary, dorsal cochlear nucleus, cricoid body, vestibular nuclei, nucleus gigantocellularis, pyrighogical area and spinal trigeminal nucleus interpolyar. Based on these data we conclude that glycinergic inputs to the trigeminal motor nucleus converge from several distinct parains of the brainstem. Funded by NIH-NIDR grant DE06193.

408.3 SPATIAL AND TEMPORAL OPTICAL RECORDING OF THE EXCITATION OF SINGLE NEURONES COMBINING A COOLED CCD AND A PHOTODIODE ARRAY. M.G. Rioult+, P. Gogan#, H.-R. Lüscher+ & S. Tyc-Dumont#. Dept. of Neurocybernétique cellulaire (Physiology (+), University of Bern, CH-3012 Bern, Switzerland and Unité de Neurophysiologie (#), CNRS UPR 418, F-13095 Marseille, France.

Investigations of synaptic integration in complex neurones necessitates simultaneous recording of the spatial and temporal patterns of excitation and inhibition in the entire neurone. Optical recording of the membrane potential by means of voltage-sensitive dyes (VSD) and a photodiode array (PDA) gives sufficient sensitivity but one CCD-array (CCDA) can provide the necessary high spatial and temporal resolution. However, a CCDA cannot provide information about the distance between two spikes. Preliminary evidences suggest that the presence of a conductance increase of the MN membrane areas, while the PDA provides the necessary temporal information.

408.4 ELECTRICAL INDUCTION AND MODULATION OF RESPIRATION IN VITRO. O. Hamada*, T. Iwahara*, E. Garcia-Rull and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Recently, we described the ability to induce locomotion using electrical stimulation of the spinal cord in cultured, isolated spinal cord segments from neonatal rats. This work investigated the mechanisms responsible for pudendal reflex suppression during micturition. Intracellular recordings were obtained from antidromically identified external urethral sphincter (EUS) and external anal sphincter (EAS) motoneurons in decerebrate male cats. Scaalfferents from the second sacral spinal cord level and the superior perineal cutaneous nerve were electrically stimulated at < 5 V (to minimize stimulus to elicit a cord dorsum potential) to produce postspontaneous potentials (PSPs) in the pudendal MNs during reflexes evoked by perineal or bladder evoked excitation. A brief addition of hyperpolarizing short pulses were injected into the MN to assess MN membrane conductance changes before and during the void. The membrane potential of EUS MNs was hyperpolarized during the bladder contraction and void while EAS MNs depolarized or showed no change. PSPs produced in EUS MNs from both peripheral nerves were reduced in amplitude or abolished when the bladder pressure was elevated and voiding occurring. Preliminary evidence suggests that the presence of a conductance increase of the MN membrane areas, while the PDA provides the necessary temporal information.

The membrane potential of EUS MNs was hyperpolarized during the bladder contraction and void while EAS MNs depolarized or showed no change. PSPs produced in EUS MNs from both peripheral nerves were reduced in amplitude or abolished when the bladder pressure was elevated and voiding occurring. Preliminary evidence suggests that the presence of a conductance increase of the MN membrane areas, while the PDA provides the necessary temporal information.
408.5 A SLOW EPSP CONTRIBUTES TO BURST DISCHARGE OF TURTLE RED NUCLEUS NEURONS REVEALED BY INTRACELLULAR RECORDINGS. M. Enomoto, J. Keifer and L.C. Hock. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

The in vivo turtle red nucleus-cerebellum is a useful model in which to study the circuit and cellular mechanisms that underlie the generation of the fast bursts of discharge observed in these neurons. Recently, we have performed intracellular recordings in red nucleus neurons in the turtle brainstem-cerebellum to gain insight into the mechanisms underlying the discharge patterns observed in these neurons. Preliminary results have shown that the red nucleus neurons exhibit a pattern of burst discharge with a high frequency of action potentials, lasting for several seconds, followed by a period of silence.

Intracellular recordings were made from single red nucleus neurons using the conventional whole-cell patch-clamp technique. The neurons were identified by their characteristic discharge patterns and the presence of synaptic inputs. The results suggest that the burst discharge is generated by a combination of excitatory and inhibitory synaptic inputs to the red nucleus neurons. The burst discharge is followed by a period of silence, during which the red nucleus neurons are relatively silent. The silent period is followed by a period of intermittent discharge, which is characterized by a lower frequency of action potentials and shorter burst duration.

These findings indicate that the burst discharge in red nucleus neurons is not simply a consequence of the discharges in adjacent neurons, but rather is generated by a complex interplay of intrinsic and synaptic mechanisms. The results also suggest that the burst discharge may play a role in the neural mechanisms underlying the behavior of the turtle. Further studies are needed to determine the role of the burst discharge in the behavior of the turtle and to explore the cellular and synaptic mechanisms underlying this discharge pattern.
408.11
PHASIC MODULATION OF TRANSMISSION FROM VESTIBULAR INPUTS TO RETICULOSPINAL NEURONES DURING FICTIVE LOCOMOTION IN LAMPROPS. R. Bandler, D. Blazquez, S. M. D. & H. S. Leclercq. Centre de Recherche en Neurosciences, Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Reticulospinal neurons (RS) play a key role in the supraspinal control of locomotion in lampreys. They provide excitation to motoneurones and to spinal interneurones involved in locomotion. In turn, they receive sensory inputs from many sources. For instance, electrical stimulation of vestibular nerve afferents induces a summation of RS responses in neurons on both sides (Rakosi & Leclercq, 1989). Reticulospinal neurones showed phasic dendritic potential oscillations related to the locomotor activity with a depolarization during ipsilateral and hyperpolarization during contralateral activity in the transverse spinal cord. On top of this modality, antisympathetic responses evoked in RS neurones by bilaterally stimulating the spinal cord were phasically modulated, reaching a minimum during depolarization and a maximum during hyperpolarization of the RS neurone. Stimulation on the contralateral side induced opposite effects: responses were maximal during depolarization and minimal during hyperpolarization. No phasic changes of the post-synaptic resistance were observed in these RS neurones, suggesting that the modulatory effects are pre-synaptic. Direct stimulation of two relay interneurones (posterolateral or posterior octavomotorius) induced similar results, although the phasic modulation was slightly less. Responses to ipsilateral stimulations were minimal during depolarization while those to the contralateral side were minimal during hyperpolarization. Taken together, these results suggest that a large part of the modulation of vestibular input transmission to RS neurones takes place either at the level of the soma of relay cells and/or at pre-synaptic terminals innervating RS neurones. (Supported by Canadian MRC, FCAR and FRSQ Quebec)

408.12
THE EFFECTS OF ACOUSTIC STARTLE RESPONSE ON LUMBAR 1A INHIBITORY INTERNEURONES. L.C. Tremblay, U. of Ottawa, Canada and J. Delwaide, U. of Liège, Belgium.

The acoustic startle response (ASR) to unexpected auditory stimulation was studied in 15 healthy subjects aged 23-40 years. The ASR was elicited by a binaurally conditioning stimulus (1000 Hz 120 db tone). The audiovisual facilitation was measured on the soleus H-reflex using a lower conditioning stimulus (1000 Hz 85 db tone). The effect of ASR on soleus was measured from 50 to 300 ms. The effect of ASR on l1 was elicited the same way as the soleus H-reflex protocol but preceded by a subthreshold electrical stimulus on the common peroneal nerve. The maximal effect of audiovisual facilitation was at a 100 ms delay 222.9% ± 67.5 expressed in percentage of H-reflex control (P<0.001). The audiovisual facilitation on l1 was significant at delays of 100 and 125 ms. The inhibition was facilitated by 20% ± 10 and 18% ± 6 (P<0.05) respectively. These results on the ASR confirm the time course and variability of audiovisual facilitation on lumbar LMS. The ASR facilitates the lumbar 1a at delays of 100 and 125 ms post-auditory stimulation. At this time only the activation of the nucleus of gigantocellularis of the reticular formation can explain the facilitation effect on l1.

408.13
ANTIDROMIC DISCHARGE OF SINGLE UNITS IN DORSAL ROOTS AND HINDLIMB CUTANEOUS NERVES. A. Niechaj, L. LaBella and S. Rossignol. Centre de Recherche en Neurosciences, Univ. de Montréal, Montréal, Québec, Canada, H3C 3J7.

Our previous work on presynaptic mechanisms during fictive locomotion has indicated that some cutaneous and muscle primary afferents, recorded intracellularly at the dorsal root entry level, may discharge rhythmically. In the present series of experiments, spike-triggered averaging (STA) was used to show that action potentials in peripheral cutaneous nerves may be propagated antidromically (AD) in such preparations.

Experiments were performed on 21 spinal cats, 15 of which were spinalized 7-22 days prior to spinalization. Single spikes recorded from peripheral nerves by bipolar electrodes were used to trigger an average of the mass activity recorded from the same root with a second electrode and digitized at 20 KHZ. Similar methods were used in the preparation without STA in the medulla where we recorded from the lateral sural (L5) nerves. Single units were found to discharge tonically in both intact and cut dorsal roots. Such antidromic discharges could last for several minutes. In a few cases, these units were rhythmically active during fictive locomotion induced by Naloxone and DOPA. AD discharges were also found in 17 LS and 10 MS recordings and were also shown in 7 semi-chronic spinal preparations in which the spinal cord was not opined in order to avoid spine trauma or changes in the temperature of the spinal cord. In a few cases, a weak modulation of such peripheral cutaneous units could be shown during fictive locomotion. It is concluded that, in such acute preparations with or without fictive locomotion, single primary afferents, recorded both in cut or intact dorsal roots, as well as in peripheral cutaneous nerves, may discharge antidromically, either tonically or rhythmically. (Supported by the MRC and by the FRSQ)

408.14

Recently, we have examined the role of the premotoneuronal spinal areas in organizing posture and movement in spinalized frogs. Our investigations revealed that microstimulation of a single site in the upper and middle layers of the frog spinal cord, in conjunction with position of the leg in the different workspaces, located a force field with a single equilibrium point. The equilibrium point is that spatial location at which the leg would be at steady state were it free to move. In the work described here, we have examined the spatial distribution of the convergent force fields (CFs) in the lumbar cord.

We investigated whether the areas that specify different equilibrium-limb positions are topographically organized. After sampling the pretomyar area of the lumbar cord, we concluded that there are at least four areas from which four distinct types of CFs are elicited. These regions form stripes oriented rostro-caudally. Within each region, a qualitatively similar set of x and y forces are produced. We also found that the simultaneous stimulation of two different areas in the area we have outlined results in a force field proportional to the sum of the fields obtained by the stimulation delivered at each point. This result is surprising given the complex nonlinearities that characterize the interactions both among neurons, and between neurons and muscles. We view a superposition mechanism as the simplest way to explain how the spinal cord generates a vast repertoire of force fields from the limited variety of available fields. This work was supported by NIH grants S595343 and AR26710, and ONR grant N000149-0372.

408.15
DIFFERENTIAL EFFECTS OF SPINAL TRAUMA IN PRIMATES AND CATS. M. Javchzad* and V.E. Amassian. Dept. of Physiology, SUNY-Health Science Center at Brooklyn, Brooklyn, NY 11208.

We demonstrated changes in direct impulse conduction in the dorsal columns (DC) and corticospinal tracts (CT) following a midlineotomy (T6) in freely anesthetized cats (J Physiol Lond 360, 1985). The DC function was assessed by recording the posterior tibial nerve responses monosynaptically stimulated at C1 or T3. A second epidural stimulus was also applied at T10 below the trauma zone and these responses were used to correct (normalizer) for any local changes at the recording site. At 8-29 hrs, DC population responses showed an immediate conduction loss after submaximal trauma, followed by a recovery phase of 30-60 min and subsequent secondary delay beginning within 2-3 hrs; the normalization process increased the definition of kinetic changes in all the models used. Following trauma, both sensory and motor systems showed transient shifts, decreased amplitudes, and selective conductive loss of the fastest elements of the population responses. In the motor system this sensitivity was seen in population studies of antidromically excited single units recorded from both motor cortex; the shortest latency units, presumably reflecting primary motor cortex, were the most affected. In the cat the DC conducted responses were less vulnerable, recovered faster and to greater extent as the CT. Extending the same paradigm to primate stimulation of two deprived and secondary delayed secondary delay took longer to develop. However, unexpectedly, the DC conducted responses in primates were less affected than the direct CT response (recorded from the pyramidal to antidiromic stimulation at T10). This finding cautioning against extrapolating results derived from models in lower forms to primates.

408.16
FORELIMB MOTOR PERFORMANCE FOLLOWING CERVICAL SPINAL CORD CONTUSSION INJURY, DORSAL COLUMN LESION, OR DORSOLATERAL Funiculus Lesion in the Rat. G. W. Schrimsher and J. J. Fleige. Dept. of Neuroscience, Univ. of Florida, Col. of Medicine, Gainesville, FL 32610.

The focus of this study is to compare the effects of weight-drop contusion injury with either selective dorsal column (DC) or dorsolateral funiculus (DLF) lesions at the C4 spinal segment on the following behavioral tests: forelimb reaching and pellet retrieval, vibrissae-induced forelimb placing, and forehead adhesive sticker removal. Control groups were present: stepping a drop 10 gms weight from a height of 2.5 cm onto a 2.5 mm tip diameter impounder resting on the exposed drus. Of 13 rats receiving this injury, 9 failed to recover reaching ability, 3 recovered with a reduced ability, 1 with a reduced retrieval rate, and 1 rat approximated normal performance levels. The inability to contact an adhesive sticker placed on the forehead or a greater than 50% drop in forehead placing on one week post-injury was predictive of permanent failure to recover reaching ability. The DC corticospinal tract was severely disrupted in all animals, regardless of whether or not reaching ability recovered. Animals that recovered reaching ability were found to have a greater sparing of lateral column white matter as compared to animals which failed to recover. Initial results from the surgical lesion studies revealed that reaching ability was spared and pellet retrieval performance approached normal levels even when the DC lesion disrupted the DC corticospinal tract. Confined bilateral DLF lesions, however, produced a profound grasp deficit involving a prominent loss of digit fixation. Lesions which affected reaching ability were found to have a marked hypomyelination in forelimb extension during reaching attempts. These findings indicate possible roles for the rubrospinal tract and the small DLF component of the corticospinal tract in performance in the rat. This work was supported by PHS Award MH15737-12.
408.17  
EFFECTS OF RETICULOSPINAL INPUT ON MONOSYNAPTIC REFLLEXES IN PARTIAL SPINAL CORD INJURY. W.H. Lee*, Y.G. Park, A. Chee, J.H. Kim, The Miami Project to Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami, FL 33136  
Rats with a spinal cord (SC) lesion sparing only the ventral quadrant at T6 level recovered coordinated fourlimb locomotion within 2-3 weeks. The time course of the functional recovery of the hindlimb movement is temporally correlated with recovery of reticulospinal nuclei (RtN) input to the lumbar SC. However, the physiological role of RtN in motor function is not clear. The objectives of this study was to investigate effects of RtN activation on monosynaptic reflex (MSR) in the normal and chronically injured SC. Sprague-Dawley rats were used. Under sterile conditions, SC lesions were made at the T6 level sparing one ventral quadrant. Rats survived from one day to ten weeks. During terminal experimentation, (a) hindlimb phasic reflexes to L4-S1 SC. Left and right L4 dorsal roots (DR) were freed and mounted on two pairs of bipolar stimulating electrodes. MSR was produced by stimulation of DR or by a conditioning stimulus. Chronic changes in the RtN inputs to the SC were estimated by changes in MSR due to RtN conditioning stimulus. Immediately after partial SC lesion, both MSR and the RtN evoked outputs were depressed on the completely lesioned side of the cord. Within 2-3 days dramatic increases in MSR appeared on the left side and peaked within one week after the lesion. Following the lesion, the excitatory effect was completely abolished on the left, whereas it remained intact on the right where the ventral quadrant was spared. (Support: NIH NS28059, The Miami Project Research Fund)

408.18  
We have shown (Goldberger & Ziegler, 1988, Soc Neurosci) that, after unilateral dorsal rhizotomy, L1-2, the deafferented hindlimb recovers quadrupedal locomotion overground and on a treadmill, but not bipedal treadmill locomotion. In order to test the hypothesis that this failure might be due to the presence of descending inhibition, the spinal cord was transected at L1 in 3 cats with chronic hindlimb deafferentation in order to abolish descending inhibition and perhaps bring back bipedal locomotion. The deafferented hindlimb, however, does not recover bipedal locomotion and even the stepping responses of the afferented limb recovered slowly and were permanently weaker than in transected-only cats. Speculating that pattern generating neurons of the afferented side were unable to activate those of the deafferented side, we examined c-fos expression in one animal after the chronic combined lesion and treadmill exercise. C-fos was expressed in interneurons on both sides of the cord. The results, taken together suggest that deafferentation reduces critically the input to spinal pattern generators and that even though neurons are activated, activation is inadequate to mediate locomotion unless the influence of descending input is permitted (or specific drugs are used). Supported by NS 228081.

408.19  
Although the NTS area has been shown to be involved in the central control of swallowing, it is unknown whether this area merely initiates the pharyngeal swallow or is involved in the motor programming of the pharyngeal swallow, as well. The purpose of this study was to examine the effect of small lesions in the NTS on the physiology of the oropharyngeal swallow in awake cats through the use of simultaneous videoradiography (VRG) and electromyography (EMG). Hooked wire EMG electrodes were implanted into six muscles (posterior tongue, mylohyoid, thyrohyoid, thyroarytenoid, inferior constrictor, cricopharyngeus) active during swallowing. Sites in the NTS area near the level of the obex that elicited swallowing upon electrical stimulation were stereotactically identified and then lesioned by passing anodal DC current. Cerebally stained neurons revealed similarly placed lesions in the ventromedial portion of the NTS and surrounding area, near the obex. Following the lesions, VRG revealed that two of the cats exhibited difficulty initiating pharyngeal volumes only. Intra-oral bolus transport was not affected. Post-lesion changes in laryngeal elevation, a pharyngeal stage event, were also found for all three cats. Although amplitude and duration measures of EMG were affected by lesioning, the sequence of EMG burst was not affected in any of the cats. Thus, the results of this study support the assertion that the ventromedial NTS and immediately surrounding area is not only involved in the initiation of the pharyngeal swallow, but may be involved in programming the duration and amplitude of pharyngeal stage events. Although these results are preliminary, small lesions to the NTS do not appear to affect the sequencing of EMG activity during the pharyngeal stage of the swallow.

CONTROL OF POSTURE AND MOVEMENT I

409.1  
The aim of this study was to determine whether vestibular afferent input are critical for triggering the rapid postural responses that are evoked by movements of the support surface under the freely standing cat. Four cats were trained to stand quietly on a moveable force platform. The animal's stance was monitored in the three lower limb muscles with increasing magnitude of head acceleration on the support surface in each of 16 different directions in the horizontal plane. The postural responses were quantified in terms of the 3-D ground reaction forces and the velocity and acceleration of the center of mass. The results, taken together suggest that the occurrence of tilt-evoked responses by foot/ankle somatosensory inputs interacting with the vestibular afferent system. Seven young normal subjects were blindfolded and fixed on a tilting apparatus. They underwent sudden forward tilt of 15° from the vertical position as acceleration increased to 3.7 and 1.8 m/s², and at 1°/s to 1.8 g with, or without passive ankle dorsiflexion. Acceleration was measured with a linear accelerometer mounted on a dental bite, and ankle position with a goniometer. Surface EMG was recorded from the temporalis anterior (TA), medial gastrocnemius (MG) and biceps femoris (BF) muscles. Our results showed that the number of responders to tilt with or without ankle rotation and the frequency of occurrence of tilt-evoked responses in the respondents, increased progressively in the three lower limb muscles with increasing magnitude of head acceleration. With the addition of ankle rotation, the only change observed was in the relative onset latencies of the two extensor muscles (MG and BF). More specifically, with ankle rotation, the proximal muscle (BF) was activated before the distal one (MG) in 4 of 5 subjects; while with ankle rotation, this pattern was reversed (MG before BF) in 5 of 6 subjects. These preliminary results suggest that the occurrence of tilt-evoked responses is dependent on the intensity of the vestibular stimulation and that the patterns of muscle activation in the lower limb muscles could be subject to modification by somatosensory inputs interacting with vestibulospinal influences.
409.3 MECHANISM OF T_{D} INDUCED SLOW DECAY OF CAT SPINDLE IA RESPONSE TO STRETCH. M. Huguenin, B. Wang*, M.S. Tabel, and E. Otton*. Dept. of Clinical Neurosciences, Univ. of Calgary, Calgary, Alta., Canada T2N 4N1, and Dept. of Neurobiology, Univ. of Groningen, NL-9712 KZ Groningen.

The sensitization of spindle IA response to stretch during dynamic fusimotor activation has been linked to stretch induced release of bag I fibers, and the slow decay of IA firing following dynamic stretch to a slow decay of stretch activation (Boyd, O.J. Exp. Physiol. 61: 203, 1976). However, experimental assessment of this hypothesis has been inconclusive (Dickson et al., Prog. Brain Res. 80:9, 1989), leaving open the possibility that IA sensitization is due to other mechanisms, including activation of unusual visco-elastic properties of bag fibers.

Acute experiments in vivo and in vitro muscle preparation were combined with mathematical modeling of spindle receptor mechanisms (Schafalna et al., J. Neurophysiol., to press) to explore the nature of the slow decay. Tracopseudal stretch (8 mm) of the parent muscle was combined with quick release (at the end of the dynamic stretch) and with various patterns of \( T_{D} \) activation. Modelling predicted that slow decay of firing due to slow deactivation should be immune to rapid release steps, regardless of amplitude. In contrast, slow decay due to a visco-elastic force transient would be abolished by release. This was confirmed experimentally. A slowly decaying transient of IA firing (evoked by exponentially decaying rate-modulated \( T_{D} \) activation) persisted during release steps (up to 8 mm). In contrast, with tonic \( T_{D} \) activation the slow decay of IA response after stretch was completely abolished by release steps of around 4 mm.

These findings suggest that the slow decay of \( T_{D} \)-sensitized stretch response is not due to a decay of stretch activation. Moreover, modelling indicates that it is most parsimoniously attributed to - exceptional - force-velocity characteristics of activated bag I fibers.

Supported by AHPMR (Canada).


In spinal cats, studies on the initiation of the swing phase have suggested that interactive signals responsible for interlimb coordination might be maximal in midstance and mid-swing. Is there evidence, firstly, that such interactive signals are present in the fictive, centrally-generated, locomotor pattern and, secondly, that these events are involved when restructuration of the fictive locomotor cycle occurs with changes in limb position?

In high decelerate, spinalized (T13) and paralyzed cats, fictive locomotion was recorded in both forelimbs from cut nerves to long head of triceps (TriLo, shoulder retractor and elbow extensor), lateral head of triceps (TriLa, elbow extensor) and cleidobracialis (CIB, shoulder protractor and elbow flexor).

At 55% of the extensor phase, three events occur simultaneously: ipsilateral (ipsi) TrLa abruptly rises in amplitude (inflexion point), ipsi TrLa and contra CIB start to decrease in amplitude. When the ipsi shoulder is tonically protracted or retracted or when the elbow is flexed or extended, cycle duration changes little, but burst durations and amplitudes are markedly altered. However, the similarity of the ipsi TrLa inflexion point and contra CIB onset of descent is preserved with each kind of perturbation. Restructuration of the cycle is achieved by lengthening or shortening the duration of the burst components before or after these points. This pattern of cycle restructuration was seen in five cats.

The observations that the ipsi TrLa inflexion point and contra CIB onset of descent remain coupled as the cycle is restructured by a change in ipsi forelimb tonic position and that these points distinguish which burst components are modified in this restructuration suggest that these centrally-generated events may be important for interlimb coordination. (Supported by the MRC of Canada).

409.5 SPINAL MOTOR CENTER EXCITABILITY CHANGES DURING EXERCISE OF DISTANT MUSCLES AS MEASURED BY TENDON TAP RESPONSE. M. Dimitrijevic, M. Salinger, G. Vrboca*, W. B. McKay. Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

The Jendrassik's maneuver (strong volitional effort to pull apart clasped hands) will increase stretch reflex responses in muscles not involved in the maneuver. Likewise, maximal volitional contraction (MVC) of a muscle group will increase stretch responses in other ipsilateral and contralateral muscles. Simultaneous maximal volitional ankle dorsiflexion at 20, 40, 60 and 100% of MVC with visual force feedback on contralateral quadriceps tendon tap responses were studied in six adult subjects. Surface EMG responses to tendon taps delivered with a constant force by an electromyographic hammer at regular intervals were analyzed.

Spinal motor center excitability was increased during the contraction phase as well. This paradigm provides insight into the behavior of sub-components of voluntary motor control.


Suprasegmental modulation of spinal motor center (SMC) excitabilities occurs during gait, but little is known about how central mechanisms influence extensor and flexor SMC excitability prior to movement. We studied excitability changes in extensor and flexor SMCs using triceps surae (TS) and tibialis anterior (TA) H-reflexes in 18 healthy subjects during treadmill walking. Stimuli were delivered relative to heel strike at 20 ms intervals across a 500 ms window. We found that facilitation of extensor H-reflex occurred 100 to 300 ms prior to EMG activation of TS, while flexor H-reflex facilitation preceded EMG activity in TA by 60 to 100 ms. These temporal differences indicate that there is a difference in the central mechanisms modulating extensor and flexor SMCs.

409.7 SPONTANEOUS ACTIVITY IN VENTRAL SACRAL ROOTS OF ISOLATED SPINAL CORD OF THE ADULT HAMSTER WITH AND WITHOUT 4-AMINO-PYRIDINE. A.J. Dekhuijzen1, J. Bagust2, and V.R. Edgerton2.

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In vitro ventral root activity has been recorded from the lumbar isolated spinal cord of hamsters weighing 15-25 g (Bagust, J. et al, Brain Res., 479: 138, 1989). The feasibility of recording from the isolated spinal cords of larger hamsters was explored in the present study in order to expand the utility of this preparation to study long term neuromyelplasticity. Whole isolated spinal cords, bathed in artificial cerebro-spinal fluid were studied in conditions with and without 4-amino-pyridine (4-AP) and with and without Mg2+, at temperatures of 18-21 °C in hamsters weighing 56-65 g. Electrically induced and spontaneous activity was recorded from ventral roots. Although spontaneous bursting activity occurred in 4-AP/Mg2+-free conditions, 4-AP (10-5 M) increased the burst frequency from 0.25-0.50 Hz to 1.0-6.0 Hz. The burst durations found in 4-AP/Mg2+-free conditions decreased from 0.5-1.0 s, to 0.02-0.4 s in the conditions with 4-AP. It is concluded that ventral root activity can be recorded in an isolated spinal cord from a sexually mature animal.

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409.8 EFFECTS OF PRACTICE OF FAST VOLUNTARY MOVEMENTS IN DOWN SYNDROME INDIVIDUALS. G.L. Almeida*, D.M. Corcos, M.L. Latash. 1Universidade Estadual de Campinas-Incimp/Censoval Nacional de Pesquisa, Campinas, SP 13100, Brazil, University of Illinois, Chicago, IL 60612, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612.

The effects of extensive practice of aimed elbow flexions in a standardized experimental situation upon kinematic and electromyographic (EMG) variables in two young adults with Down Syndrome (DS) were studied. The protocol included a pre-test during which DS-subjects performed series of trials including isotonic flexions and extensions, and different perturbation paradigms. Subjects were delivered relative to heel strike at 20 ms intervals across a 500 ms window. We found that facilitation of extensor H-reflex occurred 100 to 300 ms prior to EMG activation of TS, while flexor H-reflex facilitation preceded EMG activity in TA by 60 to 100 ms. These temporal differences indicate that there is a difference in the central mechanisms modulating extensor and flexor SMCs.

This study utilizes time- and frequency-domain analyses to examine the firing behavior of human motor units (MUs) during time- and frequency-modulated near-quasi sinusoidal muscle forces with their index finger. Single-MU discharges and multi-unit EMG were recorded from the antagonistic first dorsal and palmar interossei for several frequencies (range: 0.25 to 5 Hz), amplitudes, and mean levels of sinusoidal muscle force. As previously reported (Iyer et al., Soc. Neurosci. Abstract 16, 1990), MU discharge rates are modulated at the frequency of the force oscillation, as indicated by a distinct component in their auto-spectra. Further, the unitary modulations show widespread correlations, as assessed by prominent coherences between single-unit and population activities. The present results extend these findings to the antagonist muscle; in addition, we find a dependence of the modulations on the frequency of the force oscillation. For both agonist and antagonist, the modulating spectral components of MUs increased in size with frequency, and for some MUs, particularly of the antagonist, such components were present only at high frequencies. The unit-to-population coherences also increased with frequency, indicating augmented synchrony (correlations) between the modulations of the MU populations. The observed increase in depth and synchrony of MU rate modulations as well as the increased modulation of the EMG serve to counteract the effects of the low-pass filtering property of muscle.

Supported by NS 2271

INDEPENDENCE OF REFLEX AND VOLUNTARY EMG MODULATION DURING SINUSOIDAL TRACKING IN NORMALS. M. Johnson, A. Krupp*, T. Elbert, Dept. of Physiological Psychology, University of Neunberg, Univ. of Munich, Germany, and Institute for Human Movement Science, University of Munich, Germany.

Stretch reflex gain and voluntary muscle activity are linked in the "automatic (reflex) gain compensation" and "servo assistance" hypotheses. Experiments are reported illustrating conditions in which the normal concomitance of reflex and voluntary EMG is altered, violating hypotheses rigorously linking reflex and voluntary muscle activation.

A sinusoidally-guided wrist tracking task was perturbed by step tone transection evoked potentials. Evoked response deflection was acquired and used to assess modulation of the stretch reflex amplitude and wrist compliance as functions of the tracked position. Behavior at odds with a rigid linkage between the reflex and voluntary control systems may be summarized as: 1) reflex gain modulation corresponding to wrist kinematics, independent of the voluntary EMG modulation and 2) a task and load specific shift of reflex gain modulation dependent on the voluntary EMG modulation. Four observations underscore these behaviors. First, servo-driven passive joint movement through the sinusoidal tracking cycle produces a normally placed, statistically significant reflex amplitude peak with undetectable voluntary EMG modulation. Second, unloaded active tracking in a subset of subjects results in a normally placed single reflex peak and bimodal peaks of voluntary EMG modulation. Second, unloaded active tracking in a subset of subjects results in a normally placed single reflex peak and bimodal peaks of voluntary EMG modulation. Third, the shift of the reflex activity corresponds to a decrease in joint compliance, and is postulated to counter destabilizing loading or increase the precision of movement. The tracking phase sequencing of the reflex and voluntary EMG in these experiments points to a flexible relationship between reflex and voluntary control systems, altered by peripheral input, external load, and task intent. Supported by EMPP Inc. and a gift from Mr. Jud Bauer.


It has previously been shown that there is voltage-dependent excitation of these cells. It is suggested that this is at least in part due to activation of NMDA receptors.

BIOMOTOR CONTROL IN YOUNG CHILDREN. J.L. Lee* and H. G.Williams. Motor Development/Control Lab., Univ. of South Carolina, Columbia, SC 29208.

Past research on biomotor control in adults indicates that the upper extremities to move as a single unit. Dynamical theory of motor control suggests that biomotor control system can accommodate systematic departures from such linkage and that such deviations depend on both task and environmental demands. The purpose of this study was to examine the deviation of biomotor control in young children and to describe conditions under which linkage of the two hands is more or less tightly linked. Movement time, reaction time, error, and accuracy of responses were analyzed using repeated measures MANOVA techniques.

Bimanual movements of both children and adults were more tightly linked when movements were to equidistant targets and in asymmetrical movements where the RH covered a longer distance. Movement times were also more tightly linked under reduced visual conditions. Errors in performance were greatest under limit-only conditions. These data suggest that both task and environment strongly influence the degree to which the upper extremities are constrained to act as a single unit.

ALTERATIONS IN POINTING AND HAND CONTROL PRODUCED BY PRISMS AND BY INTERPOSITUS INACTIVATION IN THE MONKEY. C.R. Marks, B. W. F. Banks, and J.W. Kehoe. Laboratory of Neurosciences/Dept. of Physiology, Northwestern Medical School, Chicago, IL 60611.

Accurate pointing and hand control is an important action of the neuromuscular system. To test control and adaptation we perturbed the system using either prisms or muscimol injection of the interpositus nucleus. A monkey was over-trained to make rapid reaches (time restricted reach) to a 6 by 6 array of touch detectors placed vertically in front of him within easy reach. We compared this to a task in which the monkey reached freely (free reach) for food pieces. Both tasks were performed with the head immobilized. When displacing prisms (.8 to 1.8 cm) were added, changed or removed during either task, the monkey required several trials to adapt and performance was dependent on the voluntary EMG serving functions of propulsion and cocontraction through the tracking cycle. Third, the imposition of an anti-elastic load (active position feedback) reduces the voluntary EMG by approximately 180° compared to that resulting from an elastic load, yet the reflex peak remains normally situated at maximal velocity in the direction of muscle shortening. Fourth, associated with the inherently unstable anti-elastic anti-loads and during employing a "precision" tracking strategy is an increase of reflex activity in tracking phases of homonymous muscle lengthening. This shift of the reflex activity corresponds to a decrease in joint compliance, and is postulated to counter destabilizing loading or increase the precision of movement. The tracking phase sequencing of the reflex and voluntary EMG in these experiments points to a flexible relationship between reflex and voluntary control systems, altered the peripheral input, external load, and task intent. Supported by EMPP Inc. and a gift from Mr. Jud Bauer.


Recent work (Kelso, Buchanan and Wallace, Exp. Brain Res. 1991) has identified relative phase as an organizing parameter for the coordination across multiple limb movements. Phase transitions (or bifurcations) were observed when a temporal control parameter, movement frequency, was systematically varied. In KBW (1991) we hypothesized that bifurcations may occur when a spatial parameter, forearm orientation, is manipulated. To test this hypothesis, six subjects rhythmically coordinated flexion and extension of the elbow and wrist joints of the right arm under the following three initial conditions: a) forearm supine (0° of orientation): wrist flexion with elbow flexion and vice versa; and b) forearm pronate (160° of orientation): wrist flexion with elbow extension and vice versa. Starting in (1) or (2) subjects rotated the forearm in eight 20° steps, each step consisting of 15 cycles of motion at a constant frequency of 1.25 Hz. Switching from pattern (1) to pattern (2) and vice versa was observed at a critical spatial orientation. Loss of stability appears as the chief transition when the spatial parameter, forearm orientation, is manipulated. To test this hypothesis, six subjects rhythmically coordinated flexion and extension of the elbow and wrist joints of the right arm under the following three initial conditions: a) forearm supine (0° of orientation): wrist flexion with elbow flexion and vice versa; and b) forearm pronate (160° of orientation): wrist flexion with elbow extension and vice versa. Starting from (1) or (2) subjects rotated the forearm in eight 20° steps, each step consisting of 15 cycles of motion at a constant frequency of 1.25 Hz. Switching from pattern (1) to pattern (2) and vice versa was observed at a critical spatial orientation. Loss of stability appears as the chief transition when the spatial parameter, forearm orientation, is manipulated. To test this hypothesis, six subjects rhythmically coordinated flexion and extension of the elbow and wrist joints of the right arm under the following three initial conditions: a) forearm supine (0° of orientation): wrist flexion with elbow flexion and vice versa; and b) forearm pronate (160° of orientation): wrist flexion with elbow extension and vice versa. Starting in (1) or (2) subjects rotated the forearm in eight 20° steps, each step consisting of 15 cycles of motion at a constant frequency of 1.25 Hz. Switching from pattern (1) to pattern (2) and vice versa was observed at a critical spatial orientation. Loss of stability appears as the chief transition when the spatial parameter, forearm orientation, is manipulated.

This work was supported by NIH GM 41290, BRS Grant RR 07258, and ONR Grant N00014-90-J-1822.

The tendency toward phase and frequency synchronization is ubiquitous in neurobiology, and behavior, ranging from studies in visual cortex, hippocampal learning, central pattern generation, and human sensorimotor coordination. Previous work in human [1] has shown that learning a novel coordination pattern may involve a nonequivocation process. Our laboratory, however, has found that the coordination dynamics correspond to the emergence of the top of the learned pattern (e.g., a frequency-locked bimanual coordination pattern with relative phase, \( \Phi = 3/2 \text{ rad} \), 0.5 Hz, and 3/2 rad components) as in an attractor (i.e., an asymptotically stable state) accompanied by destabilization of intrinsically unstable states (e.g., \( \Phi = 1 \text{ rad} \)).

The present study reports spontaneous transfer of learning in which the pattern symmetry (e.g., \( \Phi = 3/2 \) to \( \Phi = 1/2 \)) for the pattern learned becomes stable and attractive as well. Subjects learned a visually-specified phase relation between the hands of either \( \Phi = 3/2 \) or \( \Phi = 1/2 \); Probes of the phase diagram, the set of relative phase patterns in the interval 0 to 2 \( \pi \text{ rad} \), were conducted during the learning process. When \( \pi/2 \text{ rad} \) was the pattern to be learned, spontaneous generalization to its symmetric partner, \( 3\pi/2 \text{ rad} \), was found, vice versa. Thus, learning and transfer of relative timing patterns are governed not by specific lead-lag relations between the components, but by intrinsic dynamic constraints found on symmetry.

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409.16 RELATIONSHIP BETWEEN STIFFNESS AND NET JOINT TORQUE DURING BALLISTIC ELBOW JOINT MOVEMENT. D.J. Bennett, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Previously we demonstrated that the stiffness of the elbow joint drops to as low as \( N \cdot \text{m/rad} \) during slow arm movements. We hypothesized that these changes may be proportional to changes in net joint torque (muscle torque) used to accelerate the limb inertia, but we were unable to test this hypothesis because of the limited number of movement speed studies and the confounding influence of co-contraction induced by the perturbations. The objective of the present study was to measure the stiffness changes over a large range of movement speed and load conditions, while minimizing and controlling for the effects of co-contraction. Subjects executed ballistic movements (1 rad) with strict velocity constraints (\( < 10 \% \) of peak angular velocity) at a fixed torque motor applied a small pseudo random binary (PRBS) position perturbation (\( \leq 0.1 \text{ rad} \), servoed about the mean unperturbed trajectory. In contrast to the random torque perturbations that used previously the position perturbations did not obligate the subject to co-contraction or otherwise stiffen the joint, to remain near \( < 0.1 \text{ rad} \) the nominal trajectory. EMG recordings indicated that during the movement the reflex activity contributed to the joint stiffness, but little co-contraction was induced by the perturbations. As found previously for slow movements, the stiffness dropped during movement, with a minimum (\( < N \cdot \text{m/rad} \)) at the zero crossing in the net joint torque. This phenomenon persists even for very fast movements. After compensation for the passive stiffness, the peak stiffness was found to increase proportionally to the peak joint torque. Unlike the net joint torque, the stiffness changes were often abrupt, dropping steeply at the movement onset and rising gradually as the target was approached.

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409.17 A MODEL OF MOTOR UNIT RECRUITMENT WHICH RESULTS IN INDEPENDENT CONTROL OF EQUILIBRIUM POSITION AND STIFFNESS OF THE JOINT. D.J. Reed and D. F. Mussa-Ivaldi, Department of Neurophysiology, Emory Univ Sch of Med, Atlanta, GA 30322.

We have previously proposed separate descending cortical systems for the control of co-contraction and joint position (Hamayam and Reed, Neurosci. Abstr. 7:740, 1981). The present paper discusses a hypothetical model which could provide such control at the level of the spinal-cord. The model postulates that the motor pool is divided into two regions in the lumbar grey matter. Microstimulation of these four areas generates four distinct fields with different equilibrium points. (3) We hypothesize that the motor units are recruited according to size. (Henneman, JN 409:17, 1981). The present paper discusses a hypothetical model which could provide such control at the level of the spinal-cord. The model postulates that the motor pool is divided into two regions in the lumbar grey matter. Microstimulation of these four areas generates four distinct fields with different equilibrium points. (3) We hypothesize that the motor units are recruited according to size. (Henneman, JN 409:17, 1981).

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Although locomotion in quadrupeds and bipeds occurs largely through sequences of flexion and extension, the full repertoire of movement requires torques in three dimensions. Abraham and Loeb (Exp. Brain Res. 6, 580-593, 1985) showed that the plantar flexors (PL) and eversion (PB) of the peronei to eversion and to sagittal plane movements are not known. We have previously proposed separate descending cortical systems for the control of co-contraction and joint position (Hamayam and Reed, Neurosci. Abstr. 7:740, 1981). The present paper discusses a hypothetical model which could provide such control at the level of the spinal-cord. The model postulates that the motor pool is divided into two regions in the lumbar grey matter. Microstimulation of these four areas generates four distinct fields with different equilibrium points. (3) We hypothesize that the motor units are recruited according to size. (Henneman, JN 409:17, 1981).

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409.19 EVIDENCE THAT REFLEXES BETWEEN PLANTARFLEXORS AND EVERETERS DECOUPLE ACTIONS OF THESE MUSCLES AT THE CAT ANKLE JOINT. S.J. Bonassera* and T.R. Nichols. Department of Physiology, Emory University, Atlanta GA 30322.

It is well established that the peroneal muscles of the hindlimb are coactivated with the triceps surae during stance and locomotion (Rasmussen et al., J Morphol 155:253-270, 1978). Although the major function for ankle torque (PB) is to provide eversion and toe out at the ankle joint, gastrocnemius (G) also provides a major component of toe out and eversion at the ankle (Lawrence et al., Soc Neurosci Abst 19, this volume).

Since both PB and G act in ankle eversion, we investigated stretch-evoked reciprocal reflex interactions between the peronei and G, as well as soleus (S), tibialis anterior (TA), and other lateral and posterior compartment muscles in decerebrate cats. At low forces, reflex interaction between PB and G was asymmetrical, with PB contributing a stronger inhibition onto S. However, at high forces, mutual inhibition between PB and G was asymmetrical. Reflex interaction between PB and TA were weak. The inhibition observed between the plantarflexors G and S, and the extensor peronei may serve as a protective action of G and S from actions of PB under high force conditions and thus partially compensate for the significant eversion torque generated by G and the slight plantarflexion torque generated by PB.

(Supported by NIH grant NS20855)

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The purpose of this work is to investigate how motor outputs might be combined by the central nervous system for generating a wide repertoire of behaviors. Recent experiments in the spinal frog have shown that the focal microstimulation of the premotor layers in the lumbar grey area results in a field of forces that can be expressed as a function of the position of the limb. These observations have revealed that the neural field by measuring the isometric forces induced at different ankle locations by the stimulation of the same spinal sites. Our experiments have produced three major results. (1) The stimulation of the premotor layers induces a field of forces converging toward an equilibrium point at which the volume force is zero. (2) There are at least three regions in the lumbar grey matter. Microstimulation of these four areas generates four distinct fields with different equilibrium points. (3) We have previously proposed separate descending cortical systems for the control of co-contraction and joint position (Hamayam and Reed, Neurosci. Abstr. 7:740, 1981). The present paper discusses a hypothetical model which could provide such control at the level of the spinal-cord. The model postulates that the motor pool is divided into two regions in the lumbar grey matter. Microstimulation of these four areas generates four distinct fields with different equilibrium points. (3) We hypothesize that the motor units are recruited according to size. (Henneman, JN 409:17, 1981). We have previously proposed separate descending cortical systems for the control of co-contraction and joint position (Hamayam and Reed, Neurosci. Abstr. 7:740, 1981).

The tendency toward phase and frequency synchronization is ubiquitous in neurobiology, and behavior, ranging from studies in visual cortex, hippocampal learning, central pattern generation, and human sensorimotor coordination. Previous work in human [1] has shown that learning a novel coordination pattern may involve a nonequivocation process. Our laboratory, however, has found that the coordination dynamics correspond to the emergence of the top of the learned pattern (e.g., a frequency-locked bimanual coordination pattern with relative phase, \( \Phi = 3/2 \text{ rad} \), 0.5 Hz, and 3/2 rad components) as in an attractor (i.e., an asymptotically stable state) accompanied by destabilization of intrinsically unstable states (e.g., \( \Phi = 1 \text{ rad} \)).

The present study reports spontaneous transfer of learning in which the pattern symmetry (e.g., \( \Phi = 3/2 \) to \( \Phi = 1/2 \)) for the pattern learned becomes stable and attractive as well. Subjects learned a visually-specified phase relation between the hands of either \( \Phi = 3/2 \) or \( \Phi = 1/2 \); Probes of the phase diagram, the set of relative phase patterns in the interval 0 to 2 \( \pi \text{ rad} \), were conducted during the learning process. When \( \pi/2 \text{ rad} \) was the pattern to be learned, spontaneous generalization to its symmetric partner, \( 3\pi/2 \text{ rad} \), was found, vice versa. Thus, learning and transfer of relative timing patterns are governed not by specific lead-lag relations between the components, but by intrinsic dynamic constraints found on symmetry.

Research partly supported by NIH grant MH49900, SRSG SAS Grant NS 1-207- R07358-01, and contracts N00014-84-1-1191 from the U. S. ONR.

409.21
CONTROL OF BODY MOMENT DURING STANDING LEG FLEXION MOVEMENTS IN MAN. R. V. Rogers and Y. C. Pai. Physical Therapy, Northwestern University Medical School, Chicago, IL 60611

The organization of postural responses underlying dynamic transitions in stance support and single leg flexion movements was examined in man. Previous observations led us to hypothesize (1) an invariant structure of the impulse-moment (IM) relationship of the body center of mass over a range of flexion speeds, and (2) an intralimb and interlimb coupling of hip abductor (AB) and adductor (AD) muscle activations for the control of lateral body momentum. Eight adults stood on 2 separate force platforms and performed rapid and slower leg flexion movements. Surface EMG was recorded from the knee flexor biceps femoris and AB and AD muscles. Kinematic data was obtained via motion analysis.

The findings generally supported both hypotheses. First, the normalized time when the peak IM occurred and the absolute magnitudes of the force-time integrals for propulsion (P), braking (B) and P/B components remained constant across speeds. Second, EMG responses revealed an apparent reciprocal organization of bilateral hip AB and AD muscle pairs linking anatomically antagonistic but functionally synergistic interlimb postural muscle elements related to the control of body momentum in the frontal plane.

409.22

In our previous study of rotations about the vertical axis, longer latency, voluntary mechanisms dominated stabilization at frequencies < 1 Hz while reflexes and biomechanics contributed at higher frequencies (Keshner & Peterson, Soc. Neurosci. Abstr. 12:35). In this study, we recorded chair and head velocities, and surface ECGs from bilateral semispinalis (SEMI) and sternoclavemastoid (SCM) muscles. Seated subjects underwent pitch rotations in the dark about a horizontal, horizontal axis. A triaxial angular rate sensor and a laser pointer, affixed to a headband worn by the subjects, were positioned at the center of the head. Stirmuli were predictable time waves and a random sum-of-sines stimulus with 5 frequencies ranging from 0.35 to 3.05 Hz which should be high enough to elicit vestibulocollic (VCR) and cervicocollic responses. One condition tested voluntary activation of SEMI stabilization (VS), and the second used mental arithmetic to minimize voluntary intervention (MA). Gain and phase of head velocity and EMG responses were calculated using a best fit sinusoid and analyzed with respect to chair velocity. At 0.3-1 Hz the head was well stabilized in VS and had stabilizing responses with gain ~ 0.3 in MA. Both SEMI and SCM EMG responded in phase with chair position. At 1-2 Hz good head stability was seen in both conditions. SEMI was modulated in phase with chair velocity indicative of a VCR response but SCM continued to have a position-related response. Above 2 Hz mechanical responses dominated head motion in both conditions and large EMG responses related to this motion were recorded in SEMI and SCM. Thus, compensatory responses appeared at lower frequencies and were sustained over a greater frequency range than during horizontal rotations. EMG data were consistent with the idea that SEMI is dominated by vestibular, SCM by neck receptor input. Supported by grant NS22490.

409.23

Effects on simple reaction time of initiating unilateral vs bilateral and proximal vs distal responses were investigated in 12 college-aged females. Distal responses were from the distal bilateral index finger extension; proximal responses were unilateral or bilateral elbow flexion. Two blocks of 20 trials were obtained for each condition from the agonist muscles were demanded, rectified and smoothed before fractionation into premotor- (PMT), motor and corticospinal components. Early results show an interaction between proximal-distal and bilateral-unilateral conditions for PMT. An explanation based on differences between the neuromuscular structure and motor program demands for each condition is proposed.

409.24

The present experiment examined how kinesthetic inputs from one joint are used to trigger movement at other joints in activities requiring movement sequences. Human subjects were required to open their hands as right elbow was passively rotated. The criterion elbow angle for hand opening was fixed at 145 deg (180 deg equals full elbow extension). The elbow was rotated at constant velocities (18-85 deg/s) and from one of seven different starting angles (111-129 deg). Both velocity and starting angle were presented in random order. The results compared to control trials where the elbow starting angle was held constant, and velocity was randomly varied. The arm and hand were screened from view, but information about hand opening errors was presented after each trial.

The results indicated that there was a large and systematic bias in opening angle when starting angle was randomly varied. Hand opening occurred before reaching the target with longer movement distances (111-117 deg starting angle) and, after the target for shorter distances (123-129 deg starting angle). This finding was independent of the velocity manipulation. These results suggest that kinesthetic inputs representing absolute elbow angles are not used to trigger hand opening, rather, the movement is triggered by components of velocity and time, or the angular distance from the starting angle.

409.25
RECRUITMENT ORDER OF MOTOR UNITS IN WRIST EXTENSOR MUSCLES. K. E. Jones*, S. Riek* and P. J. Cordo. School of Physical Education and Neurosciences, Research Centre, U. of Otago, Dunedin, New Zealand.

Extensor carpi radialis (ECR) contributes to wrist extension and radial deviation, while its neighbour, extensor digitorum communis (EDC) contributes to extension of the wrist and extension of various fingers. The following study examined recruitment order of motor units contributed to extension; proximal responses were unilateral or bilateral elbow flexion. Two blocks of 20 trials were obtained for each condition. EMG from the agonist muscles was demanded, rectified and smoothed before fractionation into premotor- (PMT) and motortime components. Early results show an interaction between proximal-distal and bilateral-unilateral conditions for PMT. An explanation based on differences between the neuromuscular structure and motor program demands for each condition is proposed.

409.26

The objective of this project was to evaluate the mechanical performance of oc-casionally contracting soleus muscle under different sizes of pure inertial loads and in reflexive and afferent conditions. Specifically, our aim was to examine the dispositions of muscle, assessing its performance by using kinematic variables such as energy and momentum. Both inertial loads were simulated via motor and analog circuit using force feedback. The force was integrated twice and referred to the motor which acted as a length servo. In the first experiment, the simulated load plotted as a function of frequency corresponded exactly to a mass. In the first class of experiments, the soleus muscle with the reflex intact from a decelerated cat was attached to the motor and the cross-extension reflex was used to elicit a background of 5N. An initial stretch velocity was set and the muscle was free to move under the same starting angle as yielding a force impulse to the motor. Both the initial velocity and magnitude of the muscle could be independently specified. In the second class of experiments, the protocol was repeated except the muscle was originally inactive and then the nerve was electrically stimulated for two pulses, providing a transient condition. The results indicated that there was a large and systematic bias in opening angle when starting angle was randomly varied. Hand opening occurred before reaching the target with longer movement distances (111-117 deg starting angle) and, after the target for shorter distances (123-129 deg starting angle). This finding was independent of the velocity manipulation. These results suggest that kinesthetic inputs representing absolute elbow angles are not used to trigger hand opening, rather, the movement is triggered by components of velocity and time, or the angular distance from the starting angle.

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409.27
FUNCTIONAL CLASSIFICATION OF NERVE FIBERS VIA SCALE-INVARIANT CLUSTER ANALYSIS ALGORITHM. M. Hatipoglu*, J. Hocking, J. Marchase, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

The analysis was similar to that of Camp and Pinsker [1]: two electrodes are placed to identify initial cluster centers and the determinant of the covariance matrix is known. The sorting of the real data were met (amplitude and conduction velocity correlated, amplitudes of Type II fibers clustered and also reduced the ratio of between-cluster scatter to within-cluster scatter. The LPD filter did aid in spike detection, but it somehow reduced the number of Type II fibers clustered and also reduced the ratio of between-cluster scatter to within-cluster scatter.

The new algorithm is recommended as a way of estimating relative changes in muscle activity. We thank D. Macaluso for comments on an earlier draft. Supported by NIH grant NS41947 and NS57039.

409.28

Previously, we have described the interactions that occur between respiration, mastication, and swallowing in the analysis of swallowing. We now extend these findings by describing the interrelationships between swallowing, respiration, and mastication in the adult human.

Movements of the jaw, tongue, and larynx were recorded during spontaneous breathing and the manipulation of apple, sausage, and candy. The laryngeal signal was used as a marker for swallowing, and three characteristic patterns were investigated. The food type on most over the duration of the experiment (Kalaska et. al. 1990). Here, I will show that a normal laryngeal pattern was observed and that the larynx was used for swallowing. Overall, the patterns of respiratory and masticatory movements were consistent with the phase in which swallowing occurred.

Supported by the Canadian MRC.

409.29

The aim of this study was to measure reflex responses to a perturbation, delivered just prior to the onset of voluntary pointing movements in different directions. We expected that the EMG response to the perturbation would be systematically modulated with direction, and in particular, would be least when the intended direction coincided with the perturbation direction.

Motion of the arm was restricted to rotations about the shoulder and elbow joints in the horizontal plane, and in some trials, a force perturbation (30 mN) was unexpectedly applied to the forearm, extending the elbow-joint and shoulder joint. A clear response to stretch in elbow flexors was observed over the period 30-60 ms from the start of the perturbation, and response amplitude was relatively constant after this short-latency response. The perturbation consistently elicited an earlier and faster target-reaching movement, also evidenced by earlier and larger EMG activity in the agonistic and antagonistic muscle pairs respectively. The perturbation was delivered directly to and in the same axis as the excised tendon. In humans, vibration must be delivered through the skin mechanoreceptor signals, or tendon organs using muscle twitches produced by intrafascial stimulation. We were able to entrain these receptors (especially Ia fibers) to form a model of human skin mechanoreceptors. Cats were implanted with bipolar nerve cuff electrodes on the sciatic nerve and four bipolar intramuscular stimulating electrodes in the heads of tibial and plantar nerves. In experiments with anesthesia, the cuff was placed against the object that would occur if the crisp force was applied to the object. By 80 ms after the start of a ramp stretches delivered by the vibrator. We defined the directional tuning of cells which are characterized by a 'tuning' curve (TC) that varies as the cosine of the difference between the direction of hand movement and a preferred direction (Georgopoulos et. al. 1982; 1983; Kalaska et. al. 1989). However, some cells exhibit a TC that is too skewed to show a good fit to a sinusoid (Georgopoulos et. al. 1990). Here, I will show that a normal sinusoidal and sharply tuned and skewed TC can be created by cells which either command or are correlated with muscle velocities. The TC of these cells is calculated by computer simulation for 1 cm to 8 cm prime arm movements with a bell shaped hand velocity profile in a horizontal plane. The instantaneous firing rate is assumed proportional to the amount of movement. Muscle movement arms are kept constant to isolate the effects of arm geometry. Average firing rates are computed for different portions of the movement for different muscles and for different starting locations. The TCs that we find are consistent with results derived analytically by linearizing the arm kinematics (Mussa-Ivaldi, 1988). The TC for the instantaneous firing rates are more skewed and sharply tuned for movements larger than 5 cm. This skewing is enhanced for movements starting with the elbow more fixed for both single-joint and double-joint related cells. The TC for the average firing rate reflects these same properties: for the first half of the movement it is sinusoidal, but it can be very skewed if the average includes the last half of the movement. The amplitude of the TC for shoulder related cells increases with elbow flexion, and decreases for elbow related cells and some double-joint cells. These TC characteristics may help to subclassify movement related cells, and should be applicable to some sensory cortical cells. Since force commands may be realized by changes in rest length, a distortion of some of these TC properties may also be found in cells which influence muscle force.

409.30
THE PREDICTED DIRECTIONAL TUNING OF CELLS WHICH ARE CORRELATED WITH MUSCLE KINEMATICS. S.A. Ellison. Parkinson's Disease and Movement Disorder Unit, Roger Williams Med. Center, Providence RI 02908.

The activity of many movement related cells in primate motor cortex is characterized by a 'tuning' curve (TC) that varies as the cosine of the difference between the direction of hand movement (Georgopoulos et. al. 1982; 1983; Kalaska et. al. 1989). However, some cells exhibit a TC that is too skewed to show a good fit to a sinusoid (Georgopoulos et. al. 1990). Here, I will show that a normal sinusoidal and sharply tuned and skewed TC can be created by cells which either command or are correlated with muscle velocities. The TC of these cells is calculated by computer simulation for 1 cm to 8 cm prime arm movements with a bell shaped hand velocity profile in a horizontal plane. The instantaneous firing rate is assumed proportional to the amount of movement. Muscle movement arms are kept constant to isolate the effects of arm geometry. Average firing rates are computed for different portions of the movement for different muscles and for different starting locations. The TCs that we find are consistent with results derived analytically by linearizing the arm kinematics (Mussa-Ivaldi, 1988). The TC for the instantaneous firing rates are more skewed and sharply tuned for movements larger than 5 cm. This skewing is enhanced for movements starting with the elbow more fixed for both single-joint and double-joint related cells. The TC for the average firing rate reflects these same properties: for the first half of the movement it is sinusoidal, but it can be very skewed if the average includes the last half of the movement. 4) The amplitude of the TC for shoulder related cells increases with elbow flexion, and decreases for elbow related cells and some double-joint cells. These TC characteristics may help to subclassify movement related cells, and should be applicable to some sensory cortical cells. Since force commands may be realized by changes in rest length, a distortion of some of these TC properties may also be found in cells which influence muscle force.

£ 09.1

In the intact human, cutaneous mechanoreceptors are essential for the control of posture and movement in humans. The laryngeal signal was used as a marker for swallowing, and three characteristic patterns were investigated. The food type on most over the duration of the experiment (Kalaska et. al. 1990). Here, I will show that a normal laryngeal pattern was observed and that the larynx was used for swallowing. Overall, the patterns of respiratory and masticatory movements were consistent with the phase in which swallowing occurred.

Supported by NIH grant NS41947 and NS57039.

409.41
CONTROL OF POSTURE AND MOVEMENT IN HUMANS

409.42
OPTIMIZED STIMULATION OF MUSCLE AFFERENTS IN HUMANS WITH A SERVOCONTROLLED TENDON VIBRATOR. S.C. Clareon, P.J. Corde, D. Burke*, J.P. Hallet*. The Prince Henry Hospital and University of New South Wales, Sydney, Australia.

Tendon vibration is known to be an effective stimulus for muscle receptors. In reduced preparations, vibration can be delivered directly to the skin at the site of a receptive field. In humans, however, vibration must be delivered through the skin, perpendicular to the tendon, making it more difficult to control precisely the location of the stimulus. We have constructed a tendon vibrator for humans in which the force and displacement of vibration can be controlled, even where the tendon is not accessible. The tendon vibrator is a moving arm over short distances during active contractions and joint rotations.

We recorded the activity of single muscle afferents in the nerves supplying the tibialis anterior (TA) in humans using microcondraxography. We classified afferents as muscle spindles or tendon organs using muscle twitches produced by intrafascicular stimulation and a procedure or H 1 spindle afferents with ramp stretches delivered by the vibrator. We defined the optimal force and displacement of vibration to entrain afferents at vibration frequencies of 20, 40, 80 and 120 Hz in the passive muscle, during isometric contractions, and during passive and active changes of muscle length.

We were able to entrain these receptors (especially Ia afferents) in the passive limb as well as during active contractions. Our determination of the optimal force and displacement of vibration will allow this vibrator to be used to investigate quantitatively how proprioceptive input is used to control human movement.
410.3 TRANSIENT DYNAMICS IN MOTOR CONTROL OF PATIENTS WITH BASAL GANGLIA OR CEREBELLAR DISEASES. A. Reuter, C. Lebert1, K. Vajda, University of Montréal, Montréal, QC, G688, Station A, Montréal, QC, H3C 3P8 and Centre for Nonlinear Dynamics in Physiology and Medicine, McGill University.

Experimental observations of movement disorders including tremor and voluntary microamplitudes recorded during a simple visuomotor tracking task in patients with Parkinson's disease and in patients with lesions of the cerebellum are analyzed. The task required the patient to maintain a constant index position relative to a stationary baseline display on an oscilloscope. The displacement signal of the finger was delayed up to 1400 ms, recorded for 80 s and digitized at 102 Hz. Typically in control subjects, insertion of a time delay produces large amplitude low frequency oscillations (with a period two to four times the delay) superimposed over physiological tremor. The performance of patients with Parkinson's disease (whose tremor amplitude is large) and of patients with cerebellar disease (with more severe deficits) is characterized by the intermittent appearance of transient dynamics or by the presence of sudden transitions in the amplitude and/or frequency of the signal. The need to (1) develop new tools to characterize changes in dynamics (i.e., transitions) and (2) redefine movement pathologies following neurological degeneration, in terms of qualitative changes in oscillatory behaviors is emphasized.

This work is supported by NSERC (Canada) and FCAR (Québec).

410.4 ANALYSIS OF VARIABILITY IN KINEAMIC CONTROL IN ASSESSMENT OF LANDING STRATEGIES IN CHILDREN. J. Peland and P. McGinley. Physical, Occupational Therapy, McGill, Montreal, Que., H3G 1Y5

Landing from a knee-height jump down was studied in 10 children 7-13 yrs of age under normal vision. Subjects (Ss) led with the left leg and landed with both feet simultaneously on a rigid surface. The jumps were videotaped (60Hz) and analyzed off-line (Peak Performance). Hip, knee, and ankle angles for each subject were normalized to flight time duration, and a 95% confidence interval was obtained at 1% bin widths. For each subject, the bins were normalized to maximum variability (100%) and classified into epochs of Low (<30%), Average (30-70%) or High (>70%). From previously defined categories (Peland et al., Xih Int. Symp. Posture and Gait, pp 549-552,1990) Ss were placed into one of three developmental categories: Adult, Transitional, or Proximal to Distal. In Ss classified as adult, 2 points of modulation were identified for all three joints prior to landing: Low variability when the limb was in maximum extension, and high variability for the 30-42ms (90% of flight time) prior to landing. For the transitional group, modulation was less specific although all subjects showed some modulation during 1 of these epochs. For the Proximal to Distal group, there were no specific adjustments except at the mid of the landing epoch for 2/Ss. It is concluded that ability to modulate variability in limb position during landing approach is another important descriptor in categorization of development. Supported by NSERC and FRSQ.
COORDINATE TRANSFORMATION BETWEEN VISUAL AND MOTOR SYSTEMS: COMPATIBILITY EFFECTS BASED ON VISUAL FIELD, NOT MUSCLE SYNERGY DIRECTIONS. C.J. Heckman and C.J. Rymer. Department of Movement Science, The University of Michigan, Ann Arbor, MI 48109; Department of Psychological Science, New Mexico State University, Las Cruces, NM 88003.

Most natural reaching and manipulation take place with gaze oriented towards the limbs, but the limbs, unlike artificial tasks require humans to look at a display while the limbs are neither always within view nor aligned with the display. This is the primary reason originally used by the nervous system to select the limb movement direction necessary to move an object (e.g., a cursor) in the display? Young adult male and female subjects (n=128) performed a target acquisition involving combinations of "Control-Display", "Visual Field", and "Muscle Synergy" compatibility. Results show significant advantages in reaction and execution times and in error minimization when the required movement was in the same direction in the subject's 'virtual' visual field as was the required display motion in his or her actual visual field. This effect was independent of limb positions, muscle synergy, and control-display relationships. The results support 'visual field compatibility' is a universal principle of directional compatibility.

ALTERED MOTOR UNIT DISCHARGE PATTERNS IN SPASTIC PARETIC MUSCLES. J.J. Gemperline, D. Walk*, and W.Z. Rymer. Department of Biomedical Engineering, Northwestern University, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Department of Neurology, Michael Reese Hospital, Chicago, IL 60611.

The posterosterolateral system is affected in patients with spastic hemiparesis. In the uninvolved arm, spastic hypertonia can be reduced by deafferentation of the peripheral nervous system; however, no demonstrable effect can be obtained in the paretic arm. Patellar and tibialis anterior reflexes are increased in spastic hemiparetic patients compared to normal subjects. This study was done to determine the effects of deafferentation of the paretic arm on the spastic hypertonia.

We studied responses to magnetic cortical stimulation (MCS) in 3 patients with spastic hemiparesis. Magnets were applied to the vertex of the head with the face turned downward. The left arm with spasticity and the right arm were tested. Electrical stimulation to the median nerve was applied to the dorsal forearm just proximal to the wrist. EMG was recorded with surface electrodes from the abductor pollicis brevis and abductor digiti minimi muscles. EMG was recorded from the unaffected side of the body as a control. EMG amplitude and recruitment patterns were recorded from the affected side during magnetic stimulation of the primary motor cortex. EMG was evoked by supramaximal stimulation of the motor cortex, and the same number of stimuli was used for each stimulation site.

Changes in recruitment patterns were assessed by a computer program (SUSP) which measured the number of motor units recruited and the size of the motor units. The size of the motor units was estimated by the time constant of the muscle twitch. The time constant was calculated from the decay of the twitch after stimulation. The number of motor units recruited was estimated by the number of motor units activated during the twitch. The size of the motor units was estimated by the number of motor units activated during the twitch.

RESULTS: MCS failed to elicit motor activity in the spastic limb. This failure occurred despite the fact that the motor cortex was activated and the subject was able to move the paretic limb. This failure is consistent with the failure of electrical stimulation to elicit motor activity in spastic hemiparetic patients. The failure of MCS to elicit motor activity in spastic hemiparetic patients is consistent with the failure of electrical stimulation to elicit motor activity in spastic hemiparetic patients.

The failure of MCS to elicit motor activity in spastic hemiparetic patients is consistent with the failure of electrical stimulation to elicit motor activity in spastic hemiparetic patients. This failure suggests that the motor cortex is not able to activate the paretic muscles in spastic hemiparetic patients. This failure may be due to a lack of excitatory input to the motor cortex from the spastic muscles. This failure may also be due to a lack of excitatory input to the motor cortex from the spastic muscles. The failure of MCS to elicit motor activity in spastic hemiparetic patients is consistent with the failure of electrical stimulation to elicit motor activity in spastic hemiparetic patients.
410.15

Previous studies have shown that patients with striatal abnormalities do not benefit from a warning signal prior to a GO signal in a two-choice response time (CRT) task, or that patients are not faster in a simple RT task than a CRT task. We compared the abilities of Huntington's disease (HD) patients and normal controls (NC) on three CRT tasks offering different types of advance information: Watch, in which a warning appeared; None in which the word "Where?" appeared instead of the warning; and Push, in which a warning appeared, subjects pushed a button in response to it, and then pushed again when the GO signal appeared. Subjects also performed a simple RT task, single, in which there was one stimulus and one response button. Time between the warning and GO signal was varied from trial to trial, but trials were blocked by type of warning.

The benefit of the warning in the Watch and Push conditions was the same for HD patients and NC. Both groups responded more quickly in the Push than the Watch condition. Patients did not show a normal benefit in the single condition, while NC responded as quickly in this condition as they did in the Push condition. The results are discussed in terms of simple model of a motor output buffer.

410.17
LOWER ARM ELECTROMYOGRAPHY IN SCHIZOPHRENICS WITH TARDIVE DYSKINESIA. P. H. Vrtanovski, K. Kwon* and B. Y. Melter. 1Department of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

There are numerous descriptions of motor manifestations in schizophrenics with tardive dyskinesia (TD). The neuromuscular substrate of this impairment, however, is not well known. The aim of this study was to examine the EMG activity and antagonist muscles of the lower arm during a button-press response in a force control task. Seven schizophrenics with TD and 8 without dyskinesia participated in study. The task consisted of matching a target by generating the appropriate force with the index finger of the dominant hand, and maintaining this force for 6 seconds. There were 10 targets ranging from 5 to 560 cN in 90 quasi-randomly ordered trials of the test. The EMG was recorded from 6 extensor dig. and 6 flexor dig. superficialis. Results indicated that in most subjects a predominant EMG pattern reflected a reciprocal amount of activity along the force continuum, i.e., the antagonist activity was highest at low force targets and highest at high force targets, while the reverse held true for agonist activity. In four TD patients, the antagonist activity was proportional to that in agonist, i.e., a substantial amount of cocontraction was observed. The increase in cocontraction may represent one of the markers of motor impairment in tardive dyskinesia. (Supported by VA and MH-46630).

410.18
REACTIVE AND PREDICTIVE SCA LING OF POSTURAL RESPONSES IN PARKINSONIAN PATIENTS. E. Horak, J. Frank, J. Nutt*, C. Shupert, R.S. Dow Neurological Sciences Inst., Good Samaritan Hospital, Portland, OR 97209.

We investigated whether parkinsonian patients' postural deficits could be related to an inability to scale the magnitude of responses to center of mass (CM) displacements. Fifteen parkinsonian and elderly control subjects were exposed to backward surface displacements to determine whether they could use sensory feedback and prior experience to scale postural responses to displacement velocities or amplitudes.

Although parkinsonian patients scale postural responses, their torque responses were less sensitive to fast and large displacements and more sensitive to slow and small displacements than controls. Parkinsonian EMG responses were normal latency but EMG magnitudes saturated at 50-80% of control values for the fastest and largest displacements. Agonist burst durations were shorter with multiple bursts and antagonists were longer and earlier than controls.

Although peak center of pressure (CP) initially moved beyond the peak CM, CP was not maintained to accommodate the legacy of large perturbations. Thus, the primary postural deficit was in sustaining tonic postural force and not in reactive or predictive scaling of the initial triggered postural patterns. (Supported by NIH AG06474 and DC00205.)

410.19
MOTOR PREPARATION IN YOUNG AND OLD ADULTS. J. R. Burke 1 and G. Kamen* 1. (Motor Control, Laboratory, Indiana University, Bloomington, IN). Department of Exercise Science, University of South Carolina, Columbia, SC and 2Department of Physical Therapy, Boston University, Boston, MA.

Motor preparation was assessed in young (n = 20) and old (n = 20) adults by eliciting a right leg tibial nerve H-reflex (50% of the maximum H-reflex) prior to the onset of a voluntary response. The motor task was a right plantar flexion in response to a visual stimulus. The visual response stimulus preceded the response stimulus by 1 second. The motor preparation test intervals were incremented by 50 ms between the presentation of the visual response stimulus and the onset of the voluntary response. On each trial, a H-reflex was elicited at one of the motor preparation test intervals. H-reflexes were elicited between randomly selected trials. The results supported a delayed onset of agonist and antagonist motor neuron facilitation prior to a voluntary movement with age, as shown for H-reflex amplitude.

410.20
CHANGES IN MOTOR STRATEGIES WITH NORMAL HUMAN AGING. A.S. Buchanan, C.L. Coles, M.M. Corcoran, C.H. Chen 1, G.L. Almeida, Rush-Presbyterian St. Luke's Medical Center, Chicago, IL 60612, University of Illinois, Chicago, IL 60612, Universidade Estadual de Campinas, Incamp/Centro Nacional de Pesquisa, Campinas, SP 13050, Brazil.

Recent studies of single-joint movements in healthy young normal subjects have led to the development of a Dual Strategy hypothesis for voluntary control of single joint movements. This hypothesis posulates that movements can be performed by one of two strategies: speed-insensitive (SI) or speed-sensitive (SS). It is proposed that the SI strategy is generated by modulating the duration of the excitation pulse to the motor neuron pool while the SS strategy modulates the intensity of the excitation pulse. In light of ongoing changes in the motor unit with normal aging (r=0.21), movement time (r=0.04) and rigidity (r=0.18).

To elicit the SI strategy, subjects performed discrete elbow flexions in the horizontal plane from a stationary initial position to visually defined targets at different distances with a constant inertial load. To elicit the SS strategy, subjects made discrete elbow flexion movements over a fixed distance with changes in movement speed elicited by explicit instructions to the subject and also knowledge of results concerning their movement time. Joint angle, acceleration, and electromyograms from two agonist and antagonist muscles were measured.

Younger subjects all demonstrated both SI and SS strategies. In contrast, only three of eight older subjects exhibited both strategies. The other five exhibited SS appearing behavior for both paradigms. These findings are consistent with the loss of neuromotor control with normal aging that has been suggested to be more pronounced in older aged. The loss of motor units and changes in motor unit firing with normal aging may limit excitation pulse duration modulation necessary for generating the SI strategy.

This study was supported by NIH grants AR31389, NS28176 and NS23593.
410.22 AUTOMATIC & VOLUNTARY POSTURAL RESPONSES IN PARKINSON'S DISEASE. V.P. Panzer*, L.M. Hashler and T.H. Chase, Rush-Presbyterian-St. Luke's Medical Center and NeuroCom International, Clackamas, OR 97015. Limited success has been derived from the study of automatic postural responses in Parkinson's disease (PD); whereas more revealing, though contradictory results are reported in studies of limb movement. Therefore we used a battery of tests of automatic and voluntary postural movements to evaluate 10 PD patients and 10 age-matched controls. Automatic response tests included 6 conditions of posturography and 3 sizes of platform translation. To examine voluntary postural control, patients made single weight shifts to random targets and continuous weight shifting movements. Measures of COG displacement, response latency, movement speed and amplitude were evaluated. Automatic response latencies to translations and COG displacement were normal. In contrast, measures of voluntary movement amplitude were significantly abnormal (p<.005). Postural reaction time in single weight shifts was improved by optimal dose levodopa therapy. While automatic postural responses are in the normal range in PD, voluntary responses are abnormal and may be responsive to drug therapy.

411.1 OPTICAL REFLECTANCE IN THE CAT DORSAL HIPPOCAMPUS CORRELATES WITH SLOW ELECTRICAL ACTIVITY. G. R. Poe, D. M. Benson, S. S. Chirwa and R. M. Harper. Department of Anatomy and Cell Biology and the Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA, 90024-1763. We monitored optical reflectance at 700 nm from the dorsal hippocampus using a previously described fiber optic probe. Under surgical anesthesia, electrodes were precisely placed in the dentate gyrus, and the image probe was lowered to the surface of the dorsal hippocampus. Fine wires were attached to the side of the optic probe to record hippocampal slow electrical activity. Diaphragmatic leads were placed to record respiratory and ECG activity. To assess optical activity, images from the dorsal hippocampus were gathered at 2 second intervals in synchrony with the ECG R-wave for 2 hour periods which incorporated both sleep and waking states. Recordings of hippocampal and cortical EEG, EOG, diaphragm EMG, and ECOG were digitized synchronously with the image and recorded for sleep and wake. Images of particular EEG spectral composition (slow waves, desynchronized) were selected within each state. Images within similar epochs were averaged, then compared by subtraction and ANOVA analysis with an alpha of .05. The dorsal hippocampus exhibited spontaneous fluctuations in optical activity coincident with slow electrical waveforms. Maximal reflected activity occurred during desynchronized EEG activity from phasic REM sleep and during spindling discharge of quiet sleep. The least reflected activity was observed during desynchronized EEG associated with particular waking epochs. Supported by NS-22418. G.P. is supported by a Howard Hughes Medical Institute Predoctoral Fellowship. D.R. is supported by NIDR DE 07212.

411.2 CHOLINERGIC GATING OF AUDITORY RESPONSE IN THE HIPPOCAMPUS. R. Freedman, V. Luntz-Levyman, P. Bickford-Wimer, and L. Olson, Center for Neuroscience and Schizophrenia, Denver VAMC and Univ. of Colorado Health Sciences Center, Denver, CO 80262 and Karolinska Institute, Stockholm, Sweden. Decreased response to repeated sensory stimuli is characteristic of the hippocampus. Intervaration from the medial septal nucleus has been shown to be necessary for such gating of auditory response, which is observed in the evoked activity of pyramidal neurons in the CA3 region. The purpose of this study was to determine if cholinergic neurotransmission is critical to this effect and to identify which of the several cholinergic receptor types are involved. Sprague-Dawley rats were anesthetized with chloral hydrate, and recordings of auditory-evoked N40 responses were made from microelectrodes in the CA3 pyramidal layer. Auditory stimuli were presented in a paired-pulse or conditioning paradigm, with 0.5s interstimulus interval. In control recordings, the test N40 response was less than 40% of the amplitude of the conditioning N40 response. Cholinergic antagonists were subsequently infused into the lateral ventricle. The muscarinic antagonist scopolamine and the nicotinic ganglionic agonist mecamylamine were without effect. However, the neuromuscular antagonist curare blocked gating of the test response in a dose-dependent fashion, as did a-bungarotoxin (aBT). c-BT had no effect. a-BT infused into the lateral ventricle binds to large neurons outside the CA3 pyramidal layer. There is also binding in the stratum oriens of CA1, but little drug reaches the dentate. Some of the a-BT-labelled neurons in CA3 also stain for GABA by immunocytochemistry. The results suggest that gating of response to auditory stimuli in the hippocampus is mediated by cholinergic activation of GABAergic interneurons in CA3 through an a-BT-sensitive receptor.

411.3 FINE STRUCTURE AND CELLULAR CORRELATES OF HIPPOCAMPAL SHARP-WAVE BURSTS. Z. Horvat, R. Urioste*, J. M. Rector*, S. S. Chirwa, and R. M. Harper. Department of Anatomy and Cell Biology and the Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA, 90024-1763. To examine the distribution of dendritic spines across MS patients, we hypothesized that different forms of postural dysfunction exist among MS patients depending on the relative impairment of functional systems and postural control. In this study, we have used a battery of clinical and electrophysiological tests to assess function of three sensory systems (somatosensory, visual, and vestibular) and the corticospinal tract in 10 mild-moderately disabled, ambulatory MS subjects aged 39-52 yrs. Functional deficits were correlated with postural control abnormalities revealed by horizontal translations of a posture platform.

411.4 SPONTANEOUS AND EVOKED THETA FIELD AND THETA-RELATED CELL ACTIVITY OF THE ENTORHINAL CORTEX IN THE URETHANE-ANESTHETIZED RAT. G.T. Dickson and B.H. Bland, Beh. Neurosci. Res. Grp., Dept. Psy., Univ. of Calgary, Calgary, AB, Canada T2N 1N4. Theta (θ) is a near-sinusoidal waveform generated by the hippocampal formation and is associated with a well documented nosology of θ-related hippocampal cell activity. Retrosplenial or entorhinal theta, such as the entorhinal cortex, also generate θ but the theta-related cell activity is less well known. This study extends the classification system for hippocampal θ-related cell activity to the entorhinal cortex and to examine the control of cell and field activity by cholinergic agents. Both cellular and field activity were recorded from this region using an approach normal to its laminar structure and the effects of various cholinergic agents on both field and cell activity were monitored. Thus far, θ-on cells (i.e., those which fired more during θ) and one θ-off cell (i.e., one which fired less during θ) have been found. θ-on cells were found to have either rhythmic or non-rhythmic firing properties and to be either linearly or nonlinearly related to the frequency of θ in hippocampus. Systemic and intra-hippocampal administration of nikethamide and entorhinal θ and at a lag, θ cell discharge. Thus, the nosology of θ-related units in the entorhinal cortex and their control via cholinergic agents seems to be similar to that seen in the hippocampus.

The effects of dorsomedial-posterior hypothalamic (DMPH) stimulation and inactivation on hippocampal field activity was investigated in urethan-anesthetized rats. Hippocampal theta (θ) could be elicited with either tail pinch or electrical stimulation of DMPH in the range of 0.1-1.0 mA. Reversible blockade of DMPH by micro-injection of procaine hydrochloride abolished spontaneous, tail pinch, and stimulation elicited θ and resulted in a reduction in the power of hippocampal field activity. Frequency, amplitude, and power measures of hippocampal field activity showed progressive recovery during the post-procaine period as tested by tail pinch and DMPH stimulation. A second series of experiments investigated the effects of DMPH stimulation and blockade on hippocampal θ elicited by intra-hippocampal microinjection of carbachol. Compared to Colom, et al. (1991) where blockade of medial septum abolished carbachol elicited θ, blockade of DMPH did not abolish θ elicited by carbachol in the hippocampal formation or lower the pre-procaine power level of the field below control. Recovery, electrical stimulation of DMPH at appropriate levels resulted in an upward modulation of the carbachol-induced hippocampal θ frequency.


The effects of electrical stimulation of the locus pontis oralis (PO) and dorsomedial-posterior hypothalamic (DMPH) region, along with reversible inactivation of the DMPH with procaine hydrochloride (PRO-HCl), on the discharge rates of (θ)-related cells in the medial septum (MS)/vertical limb of the diagonal band of Broca (vDBB), were investigated in urethane-anesthetized rats. Electrical stimulation of either the PO or DMPH resulted in intense activation of cells in the MS/vDBB. The microinfusion of PRO-HCl into the DMPH abolished the activation of MS/vDBB θ-on cells produced by electrical stimulation of the PO. The spontaneous discharge rate of θ cells in the MS/vDBB showed two types of responses to reversible inactivation of DMPH. Some θ-on cells reduced their discharge rates significantly while others increased their discharge rates significantly, compared to the rates accompanying hippocampal θ conditions. Microinfusion of carbachol into the DMPH also produced intense activation of θ-on cells in the MS/vDBB which was abolished by subsequent microinfusion of atropine sulfate into the DMPH.


The role of the medial septum (MS)/vertical limb of the diagonal band of Broca (vDBB) in hippocampal theta (θ) generation and behavior was assessed using microinfusions of drugs into the MS/vDBB. Rats were chronically implanted with electrodes in the stratum molecular of the dentate gyrus and a microinfusion guide cannula above the MS/vDBB. Microinfusion of procaine-HCl (PRO) produced a bilateral reversible suppression of θ. The beginning of the PRO infusion was characterized by increased motor activity followed abruptly by an immobility state resembling catalepsy, associated with the suppression of θ. The duration of suppression was dose-dependent and recovery was associated with increased feeding. Microinfusion of carbachol also initially produced motor activity followed by long periods of immobility which were associated with θ activity at a constant dose dependent frequency. Any voluntary movements resulted in upward θ frequency shifts. Subsequent microinfusions of either PRO or atropine sulfate abolished immobility related θ activity in the case of atropine, movement correlated with re-instated. Thus, the septo-hippocampal pathway is critical for the generation of both atropine-resistant and atropine-sensitive θ and their behavioral correlates.


Recordings from entorhinal neurons, together with data on the anatomical targets and the excitatory/inhibitory nature of the input, aids in the interpretation of field potentials underlying the hippocampal theta rhythm. Extracellular recordings were taken from single or multiple entorhinal neurons in rats during the two most widely studied theta mode conditions: walking and urethane anesthesia. Cell firing in relation to the simultaneously recorded theta rhythm was studied in urethane anesthetized rats. 22/23 histologically identified cells from layer III of medial entorhinal cortex (MEC), were significantly phase-locked to the hippocampal theta rhythm with a mean phase of 5° (θ=theta phase peak). In contrast, only 8/23 layer III MEC cells in urethanized rats were phase-locked with a mean phase of 90°. The firing of the 4/8 layer III cells from lateral entorhinal cortex (LEC) in urethanized rats was significantly phase-locked to the theta rhythm with a mean phase of 134°.

In summary, the firing of one MEC cell layer II, driven at constant latency from the hippocampal theta rhythm, was reversibly abolished by cooling the supracallosal strata at the genu of the corpus callosum, suggesting that septo-entorhinal projections rhythmically excite those cells. Furthermore, the firing of LEC neurons near the negative peak of the dentate theta rhythm may be critical for the current sink activity near the hippocampal fissure. (Supported by NIH NS17025.)
11.11 INTRACELLULAR RECORDINGS OF RAT SUBICULAR NEURONS. J.S. Taube and C.W. Cotman. Department of Psychology, Dartmouth College, Hanover, NH 03755. Department of Psychobiology, University of California, Irvine, CA 92717.

The electrophysiological properties of CA1 pyramidal neurons have been well characterized. These cells send a major projection to the subiculum, which in turn projects to the prefrontal cortex. Although recent studies have determined the behavioral/spatial correlates of neurons in the subiculum and prefrontal cortex, relatively little is known about the electrophysiological properties of subicular neurons. Using the in vitro slice preparation and intracellular recording techniques, the present study was conducted to assess the basic properties of subicular neurons.

Potentials recorded from subicular neurons in response to intracellular depolarizing current pulses differed from CA1 pyramidal neurons. Following a 100 msec depolarization, these cells send a major projection to the subiculum, which in turn projects to the prefrontal cortex. Application of GABA produced a short-latency hyperpolarization when it was applied near the recording site, but a depolarization when it was applied in the apical dendritic region. These results illustrate common properties of CA1 and subicular neurons, with the exception of their response to a depolarizing current pulse.

11.12 SHORT TERM ALTERATIONS IN RESPONSES OF ENTORHINAL CORtical NEURONES DURING ACTIVATION OF SYNAPTIC INPUTS IN VITRO. R.S.G. Berger. Department of Pharmacology, University of Oxford, Oxford OX1 3QY.

The perforant path, which arises from neurons in layers II/III of the entorhinal cortex (EC) is pivotal in controlling hippocampal functions. To understand these functions it is important to know how the EC processes information which is destined for the hippocampus. The present experiments have examined responses of neurons in layers IV/V and II of the medial EC (MEC) to repetitive activation ofafferent inputs at different frequencies. Slices of EC were prepared from Wistar rats and maintained in an interface chamber. Synaptic responses were evoked in layer IV/V neurons by stimulating in the lateral EC and recorded with conventional intracellular electrodes. Repetitive layer II/III stimulation in layer VNEI of the MEC or the parasubiculum. Stimulation at relatively low frequencies (<1.0 Hz) elicited a fast, AMPA/kainate mediated EPSP in layer IV/V neurons which was often followed by a slow NMDA mediated EPSP. In layer II/III the NMDA EPSP showed dramatic enhancement during low frequency stimulation and elicited bursts of action potentials. Cessation of stimulation resulted in a rapid (1-2 min) decline of the slow EPSP to control levels. Higher stimulation frequencies usually resulted in failure of the slow EPSP. The fast EPSP was little altered by changes in frequency. Responses of layer II/III neurons were dominated by fast (GABA<sub>B</sub>) and slow (GABA<sub>A</sub>) IPSPs although an NMDA mediated depolarization often intervened. Both IPSPs showed a frequency dependent decrement and could practically disappeared at higher frequencies (> 2.5 Hz).

Similarly, the NMDA EPSP was enhanced and could elicit action potentials. These effects reversed rapidly (1-2 min). 2-AAP blocked the enhanced slow EPSP but not the fading of the IPSPs. The results indicate that the routes taken by synaptic information through the EC and therefore in dominance in the hippocampus are probably dependant on the frequency of the inputs.


In order to analyze some of the determinants of extracellular field potentials generated by hippocampal CA1 neurons upon stimulation of commissural afferents, a two-neuron model of field potential in space and time was developed. The population of neurons in the model used the Laplace equation to describe intra- and extracellular potentials, and Hodgkin-Huxley and linear cable equations to describe active and passive membrane currents, respectively. The conductances of the two-neuron model served to describe ionic current lines between sources and sinks and determine from them dynamics of extracellular field potentials. Because the field potentials at a given point in the extracellular space depends on the summation of the extracellular field potentials of all neurons of that population, the ionic current lines for individual neurons were used to deduce population field potentials.

Comparison of the thus derived waveforms with those obtained from extracellular recordings of CA1 pyramidal cells activated by stimulation of commissural fibers in intact rat hippocampi indicate that the model can predict qualitatively the changes in waveform observed as a function of: (1) location along the dendrite-cell body axis, (2) magnitude of commissural fiber stimulation, (3) efficacy of commissural-CA1 synapses, varied experimentally by induction of LTD, and (4) blockage of commissural vs. superficial to pial dentate cell bodies, varied experimentally by microiontophoresis of the quisqualic receptor agonist CNQX in the respective dendritic zones. We conclude that the two-neuron model is sufficient to describe extracellular field potentials generated by populations of neurons.

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Neuroanatomical studies of the hippocampal formation have shown that circuitry as a whole is not symmetric. Differences between the dorsal and ventral hippocampus are known to exist, but the functional significance of these differences is less well characterized. In order to analyze some of the determinants of extracellular field potentials generated by hippocampal CA1 neurons upon stimulation of commissural afferents, a two-neuron model of field potential in space and time was developed. The population of neurons in the model used the Laplace equation to describe intra- and extracellular potentials, and Hodgkin-Huxley and linear cable equations to describe active and passive membrane currents, respectively. The conductances of the two-neuron model served to describe ionic current lines between sources and sinks and determine from them dynamics of extracellular field potentials. Because the field potentials at a given point in the extracellular space depends on the summation of the extracellular field potentials of all neurons of that population, the ionic current lines for individual neurons were used to deduce population field potentials.

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11.17
THREE-DIMENSIONAL SPATIAL DISTRIBUTION OF PERFORANT PATH-EVOKED FIELD POTENTIALS IN HIPPOCAMPAL RECORDS SIMULTANEOUSLY IN vivo BY MULTIPLE ELECTRODES IN CA1 AND DENTATE. Andrew J. Nowak and Theodore W. Berger, Dept. of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pgh., PA 15260.

Excitatory and inhibitory fibers arising from the entorhinal cortex innervate the dorsal dentate and dentate granule cells, as well as the apical dendrites of CA1 and CA1 pyramidal cells. The present study examined the three-dimensional spatiotemporal distribution and density of the evoked EPSPs for more lateral recording sites in CA1, and 2) longer latency population EPSPs for more lateral recording sites in dentate. Finally, a significant disparity was found between the locations of orthodromically and antidromically driven CA1 pyramidal cells. Supported by ONR, AOSPR, MH00343.

11.18

Nonlinear system analytic techniques were used to study the effects of the a-2 antagonist yohimbine on the input/output dynamics of hippocampal dentate granule cells to perforant path stimuli in the anesthetized rat. After acute intravenous administration of yohimbine (4 mg/kg, i.p.) was administered after the first train, 30 min later stimuli were delivered. First, second, and third-order kernels describing the relationship between Δ and the amplitude of the evoked granule cell population spikes were obtained. Yohimbine produced an approximately 50% increase in the magnitude of first order kernels, (h1), which reflect the average population spike for all impulses in a train. Second order kernels reflect the influence of any pair of preceding impulses occurring Δ1 and Δ2 ms before the sampled impulse h2. These results suggest that yohimbine increases both the number of granule cells excited by a perforant path volley, as well as the relative contribution - though not the time course - of the majority of mechanisms providing feedforward and feedback modulation of granule cell activity. Supported by MO40343, MH39479, NS19068, and an MRC of Canada Postdoctoral Fellowship to MJM.

11.19
AUTONOMOUS AND RHYTHMICALLY DRIVEN THETA-LIKE OSCIL­LATIONS IN A COMPUTER MODEL OF THE IN VITRO RODENT HIPPOCAMPAL CA3 REGION. B.D. Trash, G. Busnelli and R. Miles, IBM T.J. Watson Research Ctr., Yorktown Heights, NY 10598; Center for Neuroscience, Rutgers Univ., Newark, NJ 07102; Institut Pasteur, Paris, France.

MacVicar and Tse's data. In contrast to CDPO, population O

11.20
AMPA RECEPTORS ARE INVOLVED IN EXCITATORY RESPONSES OF PREFRONTAL CORTICAL CELLS INDUCED BY STIMULATION OF THE HIPPOCAMPAL FOMATION IN RATS. F. Marzban, C.D. Tweedle, B. Frerick, J. Geinisman, P. Hudspeth, R.D. Traub, G. Buzsaki, Med. Lab. de Neuropharmacologie, INSERM U114, Collège de France, 11, Place Marcelia Berthelot, 75231 Paris Cedex 05, France.

In previous studies we have demonstrated that field CA1 and subiculum project to the prelimbic portion of the rat prefrontal cortex (PFC) and that this direct unilateral projection is excitatory and shows long-term potentiation (Jay et al., Br. Res. 505: 337, 1990; Lance et al., Neuroni.. Lett. 114: 125, 1987).

It was therefore of interest to determine whether the transmitter of the hippocampo-prefrontal cortex pathway is an excitatory amino acid and whether excitatory responses of PFC neurones induced by the hippocampal (HIP) stimulation are mediated by NMDA receptors. Extracellular single unit recordings were obtained from neurones in the prelimbic area of the PFC in urethane anesthetized rats and low frequency stimulation (1 Hz) was applied in the ipsilateral field CA1 or subiculum. The effects of iontophoretic application of agonists and antagonists of NMDA and non-NMDA receptors on PFC cells showing an excitatory response to the HIP stimulation (1 Hz; latency<18 ms) were analyzed. In 15 of these cells, the application of CNQX or DNBX (10-80 nM) which antagonized the AMPA evoked excitation, completely and reversibly blocked the HIP-induced excitatory responses. In contrast, the application of APV (5-50 μM), which antagonized the NMDA but not the AMPA evoked responses, failed to reduce the HIP-induced excitatory responses in the same cells.

These results demonstrate that the excitatory responses of PFC neurones to stimulation of the hippocampus are mediated by AMPA receptors and suggest that the transmitter of the hippocampo-prefrontal cortex pathway is an excitatory amino acid.

HYPOTHALAMUS I

412.1

412.2
OBSERVATIONS ON A NEW VESICULAR-LIKE STRUCTURE IN THE AXON TERMINALS OF POSTERIOR PITUITARY OF THE FEMALE RAT. J.E. Smith, E.J. Rusch, L.E. Kwan, G.J. Hatton, Neuroscience Prog., Michigan State University, E. Lansing, MI 48824. Large vesicle-like structures were observed ultrastructurally in the axon terminals of posterior pituitary of female rats. These vesicle-like structures are either round or ovoid, range in size from 0.5-1.9μm diameter, with an average diameter of approximately 1.3μm (typical peptidergic granules in the posterior pituitary of rats). 70% of terminals of these animals ranged in diameter from 0.1-0.25μm, and there are an average of 2.3 per terminal. They are usually not bounded by membrane, although occasionally small segments of membrane form their borders. These structures appear to contain particulate material of the same density as the small peptidergic vesicles in the terminals. Approximately 50% of terminal vesicle-like structures abut against pituicytes, while approximately 20% are adjacent to basal lamina and approximately 30% are intermingled among other terminal vesicle-like structures. The data indicate that an increase in the number of vesicle-like structures within the terminal is related to the presence of the pituitary peptide releasing hormone (PRH) in the terminals containing these structures are located in the center of the posterior pituitary and 25% are located around the periphery of the pituitaries (p<0.05, Chi Square). 70% of terminals containing these structures are located in the center of the posterior pituitary and 25% are located around the periphery of the pituitaries (p<0.05, Chi Square). Alonso and Assenmacker (Cell Tissue Res. 1981, 219:525) have shown that supraspinal nucleus sends terminals primarily to the center of the posterior pituitary, with scattered projections to the periphery, while paranventricular nucleus projects terminals only to the periphery. To the best of our knowledge this is the first report of these large vesicle-like structures in the posterior pituitary of the female rat. Supported by NS 09140 and Trgr NS 07279.
412.3
Neurosecretory results of the supraoptic nucleus (SON) manufacture, transport and secrete the neuropeptide hormones oxytocin and vasopressin. These neurons have been shown to be osmotically activated by administration of hypertonic NaCl. We examined, for the first time, changes in several morphological parameters in response to an acutely induced rise in plasma osmotic pressure. Male rats were given intraperitoneal injections of isotonic (0.15M) or hypertonic (1.5M) NaCl solution and sacrificed 5 h after injection. Electron micrographs of the SON of 14 NaCl injected rats (0, 0.15M, 8, 1.5M) were compared to determine morphological differences among Mauthner cells, magnocellular neuron somata (MNC) somata in the two conditions. In the SON cell body zone significant decreases were found in the extent of glial contact with the MNC membrane (0.15M=73%, 1.5M=57%, p<.001), amount of synaptic contact of MNC with other MNC cell bodies or dendrites increased (0.15M=7%, 1.5M=19%, p<.001). Number of synaptic contacts (one terminal contacting 2 or more cells) per 100 um of somatic membrane in the 1.5M NaCl condition was double that found in the 0.15M NaCl condition. Both size of MNC membrane increased (0.15M=10%, 1.5M=13%, p<.01); and amount of glial contact with the MNC membrane increased (0.15M=10%, 1.5M=13%, p<.01). An increase in neural occupation of the SON was observed following injection of 1.5M NaCl. These morphological changes all occurred within five hours of the injection indicating a degree of plausibly heretofore unappreciated. Supported by NS 09140 and NS07279.

412.5
The magnocellular neurosecretory cells of the adult rat supraoptic nucleus show dramatic ultrastructural synaptic and astroglial changes under several conditions of increased hormone demand. Parturition (mainly involving oxytocin release) is accompanied by increase both in contact of dendrites by multiple synapses (DMS, one presynaptic terminal contacting more than one postsynaptic dendrite) and in direct dendro-dendritic contact ("bundling") in the mother rat. We also found evidence for similar changes in the same parameters with 25 min of cold or 25 min of ether anesthesia and adrenal-medullectomy prevented glial retraction under a condition known to normally occur in SON and SON. (Supported by PHS grants DA05158 and HD00718).

412.7
A monoclonal antibody (MAB) to vasopressin (VP) was specifically used to immunostain VP containing neurons. The time course of the rapidly formed DMS following perfusion indicate they must form by astroglial withdrawal from between dendrites allowing previously synaptically occupied dendrites to extend new perinematic dendritic neurites. (Supported by NS 09140)

412.8
A T-TYPE CALCIUM CURRENT IN VASOPRESSIN (AVP) AND OXYTOCIN (OT) NEURONES IN GUINEA PIG SUPRAOPTIC NUCLEUS (SON). K. P. Eriksson, O. K. Ronnekleiv and M. J. Kelly, Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201-3098.
Single-electrode voltage clamp studies were performed in SON neurones with biocytin-filled electrodes. A T-type calcium current was characterized in AVP and OT neurones, which were identified with a double labeling procedure (Ronnekleiv et al., BioTechniques 9:432-438, 1990). The T-type calcium current was TTX-insensitive, and was blocked by 100-500 μM NiCl in a dose-dependent manner. The activation threshold was -64 ± 1 mV (N = 9), and peak current occurred within 30-50 ms after the depolarization to 0 mV. The peaks of some traces were not deflected by the positive current pulse, but in most cases they were deflected by a negative current of 200-300 μA, which was necessary for phasic firing to occur in AVP neurones of guinea pig SON. (Supported by PHS grants DA05158 and HD00718).
412.9 CONVERGENCE OF μ-OPIOID AND GABA<sub>A</sub> RECEPTORS AT AN INWARDLY RECTIFYING K<sup>+</sup> CONDUCTANCE IN HYPOTHALAMIC NEURONS: MODULATION BY ESTROGEN. M.J. Kelly, W. Luo, and O.K. Ronekleiv. Dept. of Physiology, Oregon Health Sciences U., Portland, OR 97201-3098.

The effects on neurons of the arcuate nucleus, including β-endorphin neurons, of the GABA<sub>A</sub> agonist baclofen and the μ-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) were compared in hypothalamic slices from guinea pigs (GP) and rats. Intracellular recordings (3% biocytin) were made in hypothalamic slices prepared from proestrous rats or ovariectomized GP which were treated with estradiol benzoate (EB) or oil for 24 hr. Baclofen hyperpolarized all of the cells (GP:12±2 mV, N=12; rat:8±3 mV, N=11). DAMGO hyperpolarized all of the GP (N=6) and 4 of 7 rat neurons. Both baclofen and DAMGO caused a decrease in R<sub>m</sub>. The baclofen-induced current reversed at ≈ E<sub>k</sub> and exhibited inward rectification as did the DAMGO-induced current. The response to either baclofen or DAMGO was occluded by a maximal dose of the other agonist. EB treatment caused a rightward shift in the dose-response curve for baclofen which was similar to the 3.4 fold shift in the DAMGO dose-response curve. It appears that μ-opioid and GABA<sub>A</sub> receptors open the same inwardly rectifying K<sup>+</sup> channels and that estrogen alters a component (G-protein, channel) that is activated by and common to both of these receptors. (PHS grant DA05158).

412.10 INTRINSIC MEMBRANE CURRENTS IN GUINEA PIG VASOPRESSIN (AVP) NEURONS DICTATING PHASIC FIRING. O.K. Ronekleiv, K.R. Erickson and M.J. Kelly. Dept. of Physiology, Oregon Health Sciences U., Portland, OR 97201-3098.

Single-electrode voltage clamp studies in AVP-immunoreactive magnocellular neurosecretory cells in the supraoptic nucleus (SON) of guinea pigs revealed that these cells displayed two membrane currents not reported in rat SON, which were associated with phasic firing. The I<sub>n</sub> was expressed in a greater percentage of AVP neurons (83%) than OT neurons (40%). The I<sub>n</sub> was blocked by 2 mM CsCl (N=24) but not by 100-500 μM BaCl<sub>2</sub> (N=8). The time constant of activation followed a single exponential and decreased at more hyperpolarized membrane potentials (700-200 ms). The I<sub>n</sub> was modulated by forskolin, an adenylyl cyclase activator, which shifted its activation range to more depolarized levels (4 of 6 AVP cells). The I<sub>n</sub> appeared to interact with a T-type calcium current by depolarizing the cell into the range of activation of the T-current, which produced a regenerative, depolarizing potential (DP). The DP was associated with phasic firing in guinea pig SON AVP neurons (N=34; x<sup>2</sup>: p=0.004, df=2), and was present only in neurons possessing a T-type calcium current. While both currents were present in oxytocin neurons, these cells did not fire phasically, suggesting that the presence of the I<sub>n</sub> and T-type calcium current are necessary but not sufficient for phasic firing to occur. (Supported by PHS DA 05158).


The SCN in rat are the neuroanatomic substrate for the biological pacemaker underlying circadian rhythmicity. This rhythmicity is expressed in vitro in the hypothalamic brain slice preparation as a daily oscillation in overall firing frequency of SCN neurons, with peak activity occurring near the middle of subjective day. The intracellular mechanisms responsible for changes in firing frequency are unknown. As these cells are small (6-15 μm), they have proved hard to study with conventional intracellular techniques, and few such studies exist. We have begun examining these cells using whole cell patch recording in slice. Typically, these cells had resting potentials -40 to -60 mV, after-hyperpolarization (AHP) 5 to -15 mV, and overshooting action potentials of 55 to 85 mV amplitude. Our results also indicate that at least some SCN neurons have an inward rectifier current and many exhibit post-inhibitory rebound (PIR). Support: AFSOR-90-0205 & PHS NS 22155.


Innervation patterns of the dorsal motor nucleus of the vagus (DMV) and the nucleus ambiguous (NA) to the nodose ganglion (NG) from the liver and the pancreas were studied by a fluorescent double labeling technique in normal (N=5) and spontaneously diabetic BB rats (N=10). Under pentobarbital-chloral-hydrate anesthesia, the cut end of each hepatic and pancreatic nerve was marked with biocytin, Diamidino Yellow and fluorescent latex beads by retrograde diffusion. Blue, Diamidino Yellow and fluorescent latex beads) were injected in the thalamus. In order to elucidate the collateral organization of the paratrigeminal nucleus (PTI) and the known visceral and somatic afferent input to the nucleus support the idea that the PTI plays a role in somatovisceral reflexes. Supported by MRC of Canada, Grant MT-7369.

413.2 COLLATERAL ORGANIZATION OF PARATRIGEMINAL AND SPINAL TRIGEMINAL NUCLEUS PROJECTIONS: A FLUORESCENT RETROGRADE TRACER STUDY IN THE RAT. D.W. Saxon and and D.A. Hoising. Department of Anatomy, Dalhousie University, Halifax, NS B3H 4H7.

The paraganglial islands (PTI) and the marginal layer of the spinal trigeminal nucleus pars caudalis (SpVI) have complex efferent projections to the ipsilateral nucleus of the tractus solitarius (NTS), ipsilateral parabrachial nucleus (PBN) and contralateral ventrobasal complex (VBC) of the thalamus. In order to elucidate the collateral organization of the PTI and SpVI in the rat, fluorescent retrograde tracers (Fluo-gold, True Blue, Diamidino Yellow and fluorescent latex beads) were injected in combinations into the NTS, PBN and VBC. Injections into the rostral NTS produced labelled neurons throughout the ipsilateral PTI and SpVI. After injections in the VBC, labelled neurons were located predominantly in the caudal part of the PTI while after injections in the PBN they were dispersed throughout the PTI and were more numerous . SpVI I neurons projecting to the VBC were abundant in the dorsal part of lamina I and tended to be located superficially to those projecting to the PBN. Small numbers of double labelled neurons were found in the PTI and dorsal SpVI I following combined injections into the ipsilateral PBN/NTS and VBC/PBN. The topography of afferent projections to and the organization of projections from the PTI suggest that the PTI is distinct from SpVI I. The collateralization of the PTI and the known visceral and somatic afferent input to the nucleus support the idea that the PTI plays a role in somatovisceral reflexes. Supported by MRC of Canada, Grant MT-7369.
MORPHOLOGICAL PROPERTIES OF NUCLEUS TRACTUS SOLITARIUS NEURONS RECEIVING SENSORY INPUTS FROM UPPER AIRWAY RECEPTORS. R.D. Swanson, School of Dentistry, University of Michigan, Ann Arbor, MI 48109.

Morphological characteristics of lamina nucleus tractus solitarii (NTS) neurons receiving taste and tactile information from the epiglottis and aryepiglottic folds were investigated using electrophysiological mapping, Golgi impregnations and immunohistochemical techniques. Areas of the NTS responsive to chemical or mechanical stimulation of upper airway receptors occupied about one-third of the total superior laryngeal nerve termination area. Within this NTS region, chemical and tactile sensitivity was associated with different populations of multipolar neurons. Some of these NTS neurons processing upper airway sensory information have morphological properties similar to neurons located in rostral NTS and which process information from oral receptors. Supported by N.I.H. Grant DC00735.
413.9 DISCHARGE PROPERTIES OF MEDIAN RAPHE NEURONS IN FREELY-MOVING RATS DURING SLEEP-WAKING STATES. R.C. Verne and B. Kocsis, Center for Complex Systems, Florida Atlantic Univ.; Boca Raton, FL 33431

We previously demonstrated that median raphe (MR) stimulation desynchronizes the hippocampal EEG in anesthetized rats. Since MR lesions have been shown to produce continuous, running theta independent behavior. We propose that the MR may control the hippocampal desynchrony of quiet waking and slow-wave sleep (SWS). Recent recordings in cats have shown that somatotopic MR neurons fire at low rates in all behavioral states (i.e., <2-3 spikes/sec) and at progressively lower rates from waking (W) to SWS to REM sleep, while nonserotonergic MR neurons discharge at high rates in W and SWS and at progressively lower rates in SWS (4-5 spikes/sec) and relatively high rates in REM sleep (9-10 spikes/sec).

Adult male rats were permanently implanted with electrodes for recording the cortical and hippocampal EEG, the neck EMG and single unit activity in the MR during sleep-waking states. To date we have sampled the activity of 18 MR neurons; 17 of the 18 cells exhibited discharge characteristics of nonserotonergic MR neurons. The majority of non-5-HT cells fired at very high spontaneous rates during the waking state (i.e., >30 spikes/sec). Of the non-5-HT cells analyzed across all behavioral states, three populations emerged: (1) cells that fired at approximately equivalent rates in W and REM (21.7) and low rates in SWS (11.3); (2) cells that maintained high rates in W (18.9), very low rates in REM (1.05) and low rates also in SWS (2.4); and (3) cells that discharged at high rates in SWS (29) and low rates in both W and REM (5.1). All of these cells were pyramidal cells in hippocampus. The third type of cell may be involved in controlling states of mesopontine desynchronization.

Research supported by NIH Grant 45075.

413.10 RESPIRATORY NEURONS IN THE CAUDAL MEDULLA OF THE FROG, RANA CATESBEIANA. K. Koop, S. Perry* and I. J. Heemers, Dept. of Medical Physiology, U.B., Buffalo, NY 14215

To better understand the neural mechanisms underlying the coordinated cyclic movements of buccal and lung ventilation in the frog, we have begun classifying the neuronal basis in the caudal medulla for respiratory neurons, the activity of which correlated with this rhythmic activity. Deagerated, unanesthetized, paralyzed buffalogs were used. We recorded the motor output from cranial nerves (CN V, X and XII) and the activity as the intra- or extracellular activity of respiratory neurons. In addition, the response of these neurons to stimulation of CNX and X was investigated.

We found respiratory neurons, including motoneurons and non-antidromically activated neurons, from the area in and around the motor nucleus of CNX, X and XII. Neurons which were active during both buccal ventilation (Be) and lung ventilation (L), and others active during both buccal depression (BD) and during the phase immediately preceding lung ventilation (pre), were designated Be&BD and Be&BD pre neurons, respectively. Motoneurons which projected to the main branch of CNXII were of the Be&BD type, whereas, those projecting to the sternebral branch of CNXII were Be&BD pre neurons. Some Be&BD pre neurons were active (+), and were blocked by picrotoxin suggesting activation of GABAa receptors in the Be&BD pre neurons during the transition between, and a special type of L neuron with cardiac modulation. Some of the above neurons could be excited by stimulation of CNX and X. (Supported by CBF Foundation)

413.11 SYNAPTIC POTENTIALS IN MESOPONTINE CHOLINERGIC NEURONS EVOKED BY LOCAL STIMULATION IN VITRO. C. S. Leonard and R. Sanchez, Center for Neural Science, NYU 6 Wash. Pl. NY, NY 10003.

The synaptic activation of pedunculopontine (PPT) and laterodorsal tegmental (LDT) neurons was studied with intracellular recording methods in a guinea pig brain slice preparation. Brain slices were cut in the coronal plane and local stimulation was delivered through an electrode placed at the midmedullar surface, ventral to the pontine tegmentum. Cholinergic neurons were identified by combined intracellular injection of biocytin and histochemical staining for NADPH-diaphorase. Constant current pulses evoked a sequence of graded postsynaptic potentials (psps) consisting of a fast excitation and a slow inhibition. The slow ipsp was insensitive to picrotoxin and in the presence of DNQX it reversed at more hyperpolarized potentials than the fast ipsp suggesting that a major component is mediated by non-NMDA receptors. In addition, trains of stimuli enhanced the slow ipsp which could be evoked by stimulus trains at strengths below those necessary for fast psp's. The slow ipsp was insensitive to picrotoxin and in the presence of DNQX it reversed at more hyperpolarized potentials than the fast ipsp suggesting that a major component is mediated by non-NMDA receptors.

413.12 VOLTAGE-DEPENDENT MEMBRANE CURRENTS IN ACUTELY DISSOCIATED BRAIN STEM NEURONS OF RATS. G. Jiang, G. C. Rana and G. C. Haddad, Section of Respiratory Medicine, Dept. of Pediatrics, Yale Univ. Sch. of Med., New Haven, CT 06510.

In order to elucidate fundamental mechanisms underlying the neural control of cardio-respiratory function, it is essential to identify the inherent membrane properties of brain stem neurons. To study these properties in isolation, neurons from the hypoglossal (XII) and the ventral medullary motor nucleus of vagus (CNX, X) were freshly dissociated separately and studied under whole-cell voltage clamp. Using specific blockers, reversal potentials and ion substitutions, several membrane currents were characterized. XII neurons showed only one fast sensitive Na current and two outward currents: one sensitive to TTX (40mM) and the other to Co+ (1 mM), suggesting that there is a delayed rectifier (Kd) and an inward rectifier (Kw) in XII neurons. In contrast to XII neurons, DNX neurons had two inward currents, TTX (3 μM) and Na+ removal decreased but did not abolish the inward current. Addition of Co+ (1-2 μM), however, eliminated the remaining inward current indicating that both Na+ and Ca2+ (high threshold) currents are present in DNX neurons. One was AAP-sensitive and the other was Ca2+-but not Ca2+-sensitive. The remaining outward current was blocked by extracellular TTX (40 μM) and Ca2+ (1 mM) suggesting that Kd, Kw, Kw, and Kd exist in DNX cells. We conclude that I K1 and DNX neurons have different membrane properties and these currents underlie their different repetitive firing properties and 2) these results substantiate our previous data in slices since the same voltage-dependent conductances were also observed in isolated neurons.

413.13 RESPONSES OF RAPHESPINAL NEURONS TO VESTIBULAR NERVE STIMULATION IN THE CAT. B.J. Yeates, T. Oost and J. D. Delton, Lab. Neurophysiology, The Rockefeller Univ., New York, NY 10021.

Although vestibulospinal reflexes have long been known to exist, and to be potentially important in compensating for orthostatic hypotension (Doba and Reis, Circ. Res., 1974), little is known about the neural pathways which relay vestibular signals to sympathetic preganglionic neurons. The neural mechanisms that mediate these reflexes, as well as the considerable evidence suggests they contain many neurons which project to the thoracic intermedullary cell column (Morrison and Gebber, J. Neurophysiol., 1985). We have studied responses of raphespinal neurons in the thoracic spinal cord and raphespinal neurons in the cerebellum with the decerebrate cat that projected to the thoracic spinal cord responding to electrical stimulation of the vestibular nerve. Twenty 29 raphespinal neurons with conduction velocities between 1 and 4 ms/r was received vestibular inputs; 13 of the 20 were inhibited, and 7 were excited. Raphespinal neurons with conduction velocities > 4 ms are known to terminate in both the dorsal (laminae I, II, V, and X) and ventral horn (laminae VII) of the spinal cord contralaterally (Nordlund, 1985) and therefore are potential target of spinal involved in modulating excitability in both sensory and motor pathways.

Slowly-conducting raphespinal neurons thus appear to be a possible route through which sympathtic preganglionic neurons. In addition, rapidly-conducting raphespinal neurons may be in part responsible for the efferent effects on sensory and motor pathways in the spinal cord. Supported by NIH grants DC-05683 and NS-02619.

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413.15


We have shown that injections of the GABA-A agonist muscimol in the median raphe nucleus (MR) produce hyperactivity, a reduction of hippocampal serotonin metabolism, and an acceleration of dopamine metabolism in the nucleus accumbens. We examined here whether similar effects could be produced by stimulation of GABA-B receptors. Microinjections of 62.5-500 ng of the GABA-B agonist baclofen into the MR resulted in dose dependent hyperactivity which was immediate in onset. Injections into the adjacent dorsal raphe nucleus or the ventral tegmental area resulted in smaller, delayed, increases. The baclofen effect was stereospecific and could be eliminated by coinjection of the GABA-A antagonist 2-hydroxy-baclofen. These findings support the anatomical and pharmacological specificity of the baclofen effect. Baclofen produced only small effects on hippocampal serotonin metabolism, but resulted in a large increase in accumbens dopamine metabolism.

The current findings suggest that GABA-B receptors play a functional role within the MR.

413.16

EFFECT OF GABA ON NEURON TYPES IN GUSTATORY ZONE OF RAT NUCLEUS TRACTUS SOLITATORUS USING WHOLE CELL RECORDINGS IN BRAIN SLICES. R. M. Bradley, E. Bouchier and Wam Limel. School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.

We have used whole cell recordings in brain slices to characterize the biophysical and pharmacological properties of neurons in the gustatory zone of the nucleus tractus solitarius (NTS). Three neurons types have been characterized using a current injection paradigm which hyperpolarized and then immediately depolarized the neuron. The hyperpolarizing pulse resulted in an irregular pattern of action potentials initiated by depolarization in Type I neurons. In Type II neurons the hyperpolarizing pulse either delayed the initiation of the first action potential or resulted in a long first interspike interval in the spike train initiated by the depolarizing pulse. The hyperpolarizing pulse had no influence on the spike train initiated by depolarization of Type III neurons. When GABA (1 mM) was superfused over the slices, the resting membrane potential of all three types of neurons was hyperpolarized. This was accompanied by a fall in membrane resistance and marked reduction in excitability. These results demonstrate that GABA alters membrane properties of all three types of neurons in gustatory NTS. Supported by N.I.H. Grant DCOOG288.

413.17


As part of ongoing work to study the role of the Koelleier-Fuse (KF) nucleus in pain modulation a slice preparation was developed to define cellular characteristics of KF and parabrachial (PB) neurons and to confirm anatomically described projections to the trigeminal system. Extracellular recordings of the activity of PB and KF neurons were carried out in rat brainstem coronal sections (400 μm). To aid in visualization of anatomic landmarks fast green was initially added to the recording chamber with the slice in static bath conditions. Once appropriate locations for recordings were determined, the slice was maintained and recordings obtained in submerged conditions. Standard extracellular techniques with both pipettes and tungsten microelectrodes were used and signals were recorded digitally for later off-line computer analysis. Stimulation was accomplished with a 50 μA twice-Nichols tungsten microelectrode. Both spontaneous and evoked unit activity was monitored.

To date, 20 cells have been examined. 15 of these were localized in KF, 3 in the external dorsal parabrachial and 2 in the external lateral parabrachial subnuclei. In 8 of the cells amplitudes ranged between 20 and 35 μV and in 12 cells amplitudes ranged from 10 to 15 μV. According to frequency pattern, 4 cell types could be distinguished: 4 showed a constant discharge frequency of 10/second; 6 of 1.5/second, 6 of 6-8/second, and 3 showed rhythmic patterns of firing. Interestingly, evoked activity could be found in only 2 of 20 cells after stimulation of the motor trigeminal nucleus. Stimulation of principal sensory trigeminal neurons had no influence on the spontaneous activity of KF or PB cells although connections between these structures have been described.
414.1
PRENATAL RISK AND CORPUS CALLOSUM DEVELOPMENT IN LANGUAGE AND LEARNING IMPAIRED CHILDREN. P.E. Cowell T.L. Jerriam, P.A. Talai and V.H. Dentener, Biobehavioral Sciences Graduate Program, Univ. of Connecticut, Storrs, CT 06269, Dept. Psychiatry, UCSD, La Jolla, CA 92039 and Center for Molecular and Behavioral Neuroscience, Rutgers Univ., New Brunswick, NJ 08901.

A longitudinal study was undertaken of children 
16-74 mo.) and adults tested on a novel task designed to measure hippocampal function. The task, called the "DNMS" (Delayed Non-Match to Sample) task, was developed to assess memory function in animals and man. The DNMS task requires subjects to learn a set of two-part oddities (e.g., "odd" followed by "even"). Subjects are presented with two lists of stimuli and must learn which list contains the odd stimulus. The DNMS task has been used to measure hippocampal function in animals, and has been shown to be sensitive to hippocampal damage in humans.

414.2

As part of a three generation family dyslexia study, 63 members from 8 different families were scanned using magnetic resonance imaging. The corpus callosum and mid-sagittal brain areas were traced in T2-weighted mid-sagittal slices. Planimetric measures were taken of the genu, splenium, total corpus callosum, and mid-sagittal brain areas. One family showed an inheritance pattern in the total normalized corpus callosum area that was suggestive of autosomal dominant inheritance: with the father and one son having a larger corpus callosum size (0.06, 0.06) and the mother and remaining 4 children having average sizes (0.05, 0.07, 0.06, 0.06). See Figure. (p=p<0.05 size)

The corpus callosum size did not distinguish between dyslexics and non-dyslexics in this family. Currently, sib pair analysis on both corpus callosum angulation and area measurements are in progress on the overall sample.

414.3

To assess the relationship of neuroanatomical and functional asymmetries, the planum temporalis (PT), part of the posterior region of the middle temporal lobe, is studied using an Atlas of the human brain. The Atlas is based on a detailed description of the cytoarchitectonic and myeloarchitectonic organization of the human brain. The Atlas provides a detailed description of the cytoarchitectonic and myeloarchitectonic organization of the human brain. The Atlas is based on a detailed description of the cytoarchitectonic and myeloarchitectonic organization of the human brain.

414.4
RADIAL ARM MAZE MEASURES DEVELOPMENT OF SPATIAL MEMORY IN YOUNG SUBJECTS WITH CEREBELLAR TUMORS. J.A. Lazareff# and E. Castro-Sierra, Dept. of Neurosurgery and Lab. of Psychoacoustic Hospital Infantil de Mexico, 06720 Mexico, D.F. MEXICO.

Children (N=4; avg. age=10:7) with tumors of different etiologies of the cerebellum were studied for their abilities for carrying out visually-spatial tasks. Performance was measured in an 8-arm radial maze. Experimental conditions were: (1) one day of search in 8 open arms, followed by (2) 15 days of forced-choice search. In condition 1, 4 of the arms were closed and the subject searched the four open arms; then, after a delay (30-120 sec.) the arms were opened and the subject searched the remaining closed arms. In condition 1, there was a developmental progression: younger children (20-40 mo.) performed at chance levels and older children (approx. 100 mo.) and adults performed at near perfect levels. In condition 2, two adults significantly improved (trials 5-8) over the first 4 days of forced choice testing, but the youngest children failed to improve (trials 5-8) in 15 days of testing. However, since all subjects performed nearly perfectly on trials 1-4 of forced choice testing, this indicates that even the youngest children can remember at least four locations for a short period of time.

414.5
USE OF AN ODDITY TASK IN CHILDREN TO ANALYZE THE SOLUTION OF DELAYED NON-MATCH TO SAMPLE (DNMS) W.H. Overman, M. Miller* and V.H. Kilpatrick*, Kogerer Psychology Department, UNC-Wilmington, Wilmington, N.C. 28403.

The DNMS task has been used to measure hippocampal function in animals and man. Infant monkeys (Bachewaller and Mishkin, Beh. Neurosci. 49, 1984) and infant humans (Overman, Ann. N.Y. Acad. Sci. 608. 1990) were studied for their abilities for carrying out visually-spatial tasks. In one study, children (16-74 mo.) and adults were tested on a novel task designed to measure hippocampal function. The task, called the "DNMS" (Delayed Non-Match to Sample) task, was developed to assess memory function in animals and man. The DNMS task requires subjects to learn a set of two-part oddities (e.g., "odd" followed by "even"). Subjects are presented with two lists of stimuli and must learn which list contains the odd stimulus. The DNMS task has been used to measure hippocampal function in animals, and has been shown to be sensitive to hippocampal damage in humans.

414.6
MEMORY SKILLS IN YOUNG SUBJECTS WITH CEREBELLAR TUMORS. J.A. Lazareff# and E. Castro-Sierra, Dept. of Neurosurgery and Lab. of Psychoacoustic Hospital Infantil de Mexico, 06720 Mexico, D.F. MEXICO.

Children (N=4; avg. age=10:7) with tumors of different etiologies of the cerebellum were studied for their abilities for carrying out visually-spatial tasks. Performance was measured in an 8-arm radial maze. Experimental conditions were: (1) one day of search in 8 open arms, followed by (2) 15 days of forced-choice search. In condition 1, 4 of the arms were closed and the subject searched the four open arms; then, after a delay (30-120 sec.) the arms were opened and the subject searched the remaining closed arms. In condition 1, there was a developmental progression: younger children (20-40 mo.) performed at chance levels and older children (approx. 100 mo.) and adults performed at near perfect levels. In condition 2, two adults significantly improved (trials 5-8) over the first 4 days of forced choice testing, but the youngest children failed to improve (trials 5-8) in 15 days of testing. However, since all subjects performed nearly perfectly on trials 1-4 of forced choice testing, this indicates that even the youngest children can remember at least four locations for a short period of time.
414.7

IMPAIRED MEMORY CAUSED BY HERPES SIMPLEX VIRUS INFECTION.
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Deficits of impaired intellectual function associated with herpes simplex encephalitis (HSE) have not been extensively investigated. Survivors of HSE have experience irreversible impairment and an inability to store and recall information. These deficits are postulated to correlate with the stereotypic post mortem temporal lobe necrosis seen in this disease. Because olfactory and limbic system structures are central to storage and retrieval of memories, injury to these areas is believed to cause behavioral and learning disabilities. Therefore, an animal model is needed to explore the correlation between memory impairment and temporal lobe pathology. We have developed a rat model that mimics the physical and behavioral aspects of human HSE and does not involve invasive delivery of virus to the brain. Fifty-one percent of rats shed virus from their nose but rarely from their eyes following intranasal inoculation with 1 X 10^6 TCID50 of syncytial forming herpes simplex virus type-1. Similar deficits of behavioral abnormalities and motor seizures. Memory dysfunctions were evaluated using a radial arm maze test to quantitate the acquisition of information of a learned task. Infected rats (N=8) performed at chance levels which was statistically different than controls (N=8). At different times post infection, rats showed histopathologic changes within the amygdala, hippocampus, and neocortex. Viral antigens were also detected within these structures by immunohistochemistry. This model will allow neuroanatomical correlation between memory dysfunction with site-specific HSV replication, and provide a system to test possible viral or cognitive enhancing properties. Supported by the Department of Veterans Affairs.

415.1

LONG TERM RETROGRADE AMNESIA ON INHIBITORY AVOIDANCE AND CONDITIONED TASTE AVERSION LEARNING TASKS BY INSLULAR CORTEX LESION. C.E. Ormsby*, A.L. Piñá and F. Bermúdez-Almeco. Instituto de Fisiología Celular, Unan. UNAM, D.F. México. 04510

Previous works have described deficits in acquisition and consolidation of inhibitory avoidance (IA), spatial learning, and acquisition of conditioned taste aversion (CTA) produced by post-training reversible lesions of the insular cortex (ICx) (Bermúdez-Almeco et al. PNAS. In press). Male Wistar rats were tested to observe the effects of ICx lesions on long-term memory of IA and CTA tasks. Twenty-six animals were trained in CTA and 48 hr later in IA, then assigned to 3 subgroups: one group (LxG) received bilateral electrolytic lesions of the ICx 48 hr after the IA training to allow consolidation, another, was sham operated the same day (ShG) and the third group served as control (CtG). All animals were tested four days later for IA, and 11 days later for CTA. Results showed that the lesion of ICx significantly impaired IA, assessed by latency and time spent in either compartment when compared with both the ShG and CtG groups. Similarly, the CTA test showed significant impairment of the Les in the recall of the aversive reaction to saccharin. These results confirm that ICx is necessary for the storage of long-term memory of previously learned avoidance conditioning.

415.2


The purpose of the present experiment was to study retrograde amnesia in mice by using a spatial discrimination task performed in 2 different 8-arm radial mazes. Animals learned sufficiently, one at a time, and every 2 weeks, 5 pairs of adjacent arms (2 in the first maze and 3 in the second one). Bilateral entorhinal cortex lesion of the entorhinal cortex (E. C.) was performed 1 hour after subjects reached the criterion on the 5th pair.

As compared to sham-operated animals, E. C. lesioned mice were severely impaired at remembering the 2 last pairs learned within 3 weeks before surgery but had normal performance on those learned beyond this time period (Retrograde Amnesia).

Animals were trained on the second maze until they maintained the criterion on the each of 3 pairs learned before surgery. They were also trained on the 4th pair not previously used. Experimental subjects learned this 4th pair at the same rate as controls but when tested 3 weeks later, a significant forgetting was observed on the 3 pairs returned post-operatively (Anterograde Amnesia).

Results confirm previous findings (Zola-Morgan and Squire, Science, 250, 288-290, 1990) and suggest that the hippocampal formation is required for only a limited period of time after learning. Beyond this critical period, normal performance would be supported by extra-hippocampal structures, thus suggesting a "translocation" of the internal representation. In addition, as shown by additional behavioral data, it seems that a change in the code of its representation might occurred. This research was supported by Centre National de la Recherche Scientifique and by the Fondation pour la Recherche Médicale Française.

415.3

INTRACELLULAR RECORDINGS FROM NEURONS IN THE FRONTAL CORTEX OF RATS PERFORMING LICKING CONDITIONING TO A LIGHT CUE. B.P. Kristensen and C.D. Moody. UCLA Medical Center, Los Angeles, CA 90024.

Male Sprague-Dawley rats were trained to lick for water when prompted by a light cue. Intracellular as well as extracellular recordings (LiO) were obtained from neurons in the frontal cortex of animals performing the conditioned behavior in response to the light stimulus. The electrophysiological characteristics of the neurons were investigated with regard to discharge pattern for all cells and further recording potentials and input resistances for intracellularly studied units, and the correlation of these parameters with stimulus, the behavioral response or the reinforcement was analyzed.

Neurons showing increased discharge during and preceding licking were found. Intracellular recording disclosed depolarization of the baseline of licking and hyperpolarization at the cessation of licking. (Supported by HD05958)

415.4


Several investigators have suggested that the nucleus accumbens mediates the effects of the instrumental reinforcer and, as such, is involved in the control of instrumental performance. This hypothesis was assessed by examining the effects of bilateral lesions of the nucleus accumbens on instrumental performance of hungry rats in a free operant situation. In support of this proposal it was found that lever pressing responding for a food reward on a variable interval (VI) schedule was significantly impaired in lesioned animals relative to sham controls. Two potential accounts for this deficit were then examined, one associative and the other motivational. The associative account assumes that the lesion interferes with learning the response-reinforcer contingency. However, this effect could be explained by a noncontingent variable time (VT) schedule was found to produce a comparable decline in performance over trials in both the lesion and sham groups, suggesting that the lesion did not affect sensitivity to the instrumental contingency. Secondly, motivation to respond was assessed by shifts in primary motivation achieved by varying the number and value of food reinforcers prior to extinction. This manipulation induced deprivation dependent changes in performance which were, again, similar in both the lesion and sham groups. (Supported by HD05958)
415.5 ACTIVATION OF THE VENTRAL TEGMENTAL AREA AFFECTS HIPPOCAMPAL EEG. K. B. Austin, Department of Psychology, McGill University, Montreal, Canada, H3A 1B1.

Dopamine (DA) neurons in the ventral tegmental area (VTA) project to the basal forebrain, and are known to mediate brain stimulation reward. The stimulation-evoked output from the VTA and also strongly influence the electrical activity of the hippocampal formation. In intact rats, lesions of the medial septum will abolish hippocampal theta rhythm and disrupt performance on spatial memory tasks. In addition, infusions of DA into the medial septum of chloral hydrate anesthetized rats will result in the generation of hippocampal theta rhythm. In freely moving rat preparations, VTA activation using the GABA antagonist picrotxin (PTX) produces compellent, stereotyped locomotion which is accompanied by hippocampal theta rhythm. To dissociate the behavior from the EEG changes, we recorded hippocampal EEG from chloral hydrate anesthetized rats and used local injections of PTX to activate the VTA. Intra-VTA PTX (15 ng in 0.5 μl) injections produced in an increase in EEG synchrony. FFT analysis revealed an increase in EEG power in the 3-5 Hz frequency band. This theta rhythm could be abolished by increasing the anesthetic infusion rate. Subsequent applications of PTX (up to 300 ng) could not reestablish theta, however, numerous sharp-wave complexes were observed at higher doses of PTX. This data demonstrates that the hippocampal theta can be modulated by activation of the VTA. The VTA may play an important role in the generation of hippocampal theta, and, thus, this experiment represents an important step in developing the interaction between reward and memory systems in the brain.

415.6 SUBJECTIVE REWARD MAGNITUDE AS A FUNCTION OF TRAIN DURATION AND STIMULATION STRENGTH. Terry Mark* and C. R. Gallistel Dept of Psychology, UCLA, Los Angeles, CA 90024-1563.

We measured the subjective reward magnitude of electrical brain stimulation as a function of train duration and stimulation strength (where strength is defined as current x pulse frequency), using the matching law in two different ways. In the first method (trade-off method), the relative magnitudes of the rewards received from two different levels was estimated by the magnitude of the difference in the mean interreward intervals required to offset the difference in stimulation parameters. When the difference in mean interreward intervals offset the difference in reward magnitudes, the rat allocated its time equally between the levels. In the second method (direct method), the ratio of the reward magnitudes at the two levels was assumed to be directly given by the ratio of the rat’s time allocations (after correction for side bias). The results of the two methods agree. They show that: i) Reward magnitude grows steeply up to train durations of about 1 s, then saturates (stops increasing). ii) The stronger the stimulation, the steeper the growth and the higher the asymptote. iii) The saturating duration is approximately the same at different strengths of stimulation. [Supported by NSF Grant BNS 89-96246.]

415.7 Subjective Reward Magnitude as a Function of Stimulation Strength Varies Within Rats as a Function of Site of Stimulation. Matthew Leopf, C. R. Gallistel and John Liebeskind, Dept of Psychol., UCLA, Los Angeles, CA 90024-1563.

Gallistel and Leon (in press) measured the subjective magnitude of a brain stimulation reward by manipulating the mean interreward interval on one lever and determining the current or pulse frequency required on a second lever to produce equal preference between the levers. At equilibrium, the difference in the subjective magnitudes of the two rewards compensates for the difference in the mean interreward intervals. They found that the subjective reward magnitude increases approximately as a power function of the strength of stimulation (current x pulse frequency), but the power varied from 2 to 10 between subjects and/or sites of stimulation (subjects and sites were confounded). We have now determined subjective reward magnitude as a function of stimulation parameters for electrodes in the ventral tegmental area and right and left lateral hypothalamus in the same rats. We find that the exponent of the power function varies between stimulation sites within a rat, suggesting that the subjective scaling of reward magnitude is a characteristic of individual anatomical sites. [Supported by NSF Grant BNS 89-96246.]

415.9 DISCRIMINATIVE CONDITIONING OF ORIENTING HEAD MOVEMENTS IN CATS. M. Penttonen* and T. Korhonen, Dept. of Psychology, Univ. of Jyväskylä, P.O. Box 35, SF-40351 Jyväskylä, Finland.

Habitation of orienting responses to auditory stimuli has been studied quite extensively. The purpose of this study was to develop a paradigm for studying the neural mechanisms of orientation. Miniature loudspeakers were attached at a distance of 2 cm from both ears in cats. During a habituation session two tones of different pitches were presented randomly either to the left or right ear. CS+ tones were not paired with stimulation. Left or right ear CS- tones were not paired with the stimulation. The cats learned discrimination between these two tones: they turned their head significantly more to the CS+ than CS- and increased responding to the CS+ over sessions and maintained the responding at an asymptotic level during last 2 sessions. Evoked responses recorded from the cingulate cortex also showed a differentiation between discriminative stimuli. It is concluded that with this paradigm it is possible to modify orienting responses and maintain these responses at a constant level so that the analysis of underlying neural systems becomes feasible.

415.10 EFFECTS OF REPEATED MEASURES AND STIMULUS INTERRUPTIONS ON INDUCING PERSISTING HINDLIMB FLEXION (SPINAL FIXATION) IN RATS. M. J. Barbel, E.E. Johnson, and M.M. Patterson, Dept. of Psychology and College of Osteopathic Medicine, Ohio Univ., Athens OH 45701.

Stiminetz et al. ([CPP, 1981] demonstrated that persisting hindlimb flexion may be induced by 40 minutes of stimulation (2-4 mA) to a spinalized rat’s hindlimb. The present study investigated the effect of repeated asymmetrical measurements (within individual animals) and interruptions of the induction stimulus on the fixation process. If feasible, a repeated measures design would require fewer animals. Fifteen male rats received 10 trials per day for 8 days of either a 10-kHz or a 22-kHz pure tone stimulus (97dB SPL, 400ms). Animals habituated equally well to either stimulus across days. When the stimulus was switched to the other frequency on Day 9 or Day 10, no recovery was found. A subsequent same/different test of the two frequencies under varied interstimulus intervals revealed no specificity for short-term habituation. The lack of frequency specificity does not rule out either a chronic or a phasic mechanism for LTH.
415.11

INHIBITION OF PERSISTING RAT HINDLIMB FLEXION (SPINAL FIXATION) BY CONTRALATERAL HINDLIMB STIMULATION. J.J. Nett and M.G. Rush. Dept. of Psychology & College of Osteopathic Medicine, Ohio Univ., Athens, OH 45701.

Steinmetz et al., (JCPP, 1981) demonstrated that persisting hindlimb flexion may be induced by applying 40 minutes of stimulation (2-4 mA) to a spinalized rat's hindlimb. We explored the possibility that the spinal reflex alteration producing persisting hindlimb flexion could be inhibited by sensory input from the contralateral hindlimb.

Twenty-four rats were anesthetized with Nembutal (50 mg/kg i.p.) and spinalized. Twelve animals were randomly assigned to each of the experimental and control conditions and all then received stimulation (3.5 mA, 100 pps, 7 msec repetitive dc pulses) to the right hindlimb for 40 minutes. The amount of weight needed to remove the ensuing persisting hindlimb flexion was then measured. The experimental animals then received stimulation to the left hindlimb for 40 minutes while control animals received no stimulation. It was necessary to re-move persisting flexion of the right hindlimb was then again measured in both groups.

The results indicated that the contralateral stimulation appeared to lower the persisting flexion by a small (2.1 grams) but consistent and significant (p<0.01) amount. This finding suggested that the spinal fixation produced by the stimulation could be altered at least slightly by presumably the crossed extensor reflex pathways from stimulation of the opposite limb. The results suggest that there may be some possibility of reversing the persisting effects of altered reflex excistencies by utilizing opposing input.

Support: Am Osteo Ann 89-08-519

415.12


Steinmetz et al., (JCPP, 1981) demonstrated that persisting hindlimb flexion may be induced by applying 2-4 mA of electrical stimulation for at least 40 minutes to the hindlimb of spinalized rats. In 1989 and 1990 Bartell et al. (Sec. Neuroscience, 1989) found that d-amphetamine significantly increased the persisting hindlimb flexion. This study examined the effects of various doses of d-amphetamine on persisting hindlimb flexion.

Forty rats were anesthetized with Nembutal (50 mg/kg i.p.) and randomly assigned to one of four groups receiving either 0.0, 2.0, 5.0 or 10.0 mg/kg of d-amphetamine. The d-amphetamine was injected 15 min before to stimulation and the rats were subsequently spinalized at T7. Immediately following 40 minutes of 3.5 mA (100 pps) hindlimb stimulation, we assessed the amount of weight needed to remove the persisting hindlimb flexion.

The results showed an increase in hindlimb flexion which generally increased with increasing dosage. This outcome supports Bartell et al., 1989, and suggests that d-amphetamine effects may be maximal at 10.0 mg/kg. However, it may be that greater doses of d-amphetamine accentuate the flexion even more but doses greater than 10.0 mg/kg were not used due to the difficulty of maintaining anesthesia. This work further supports involvement of catecholamine in spinaflex reflexes. Further studies will evaluate specific centrally acting NE and DA agonist effects on persisting postural asymmetry. Supported by American Osteopathic Association grant 89-08-519.

415.13


The mormyrid electro sensory lobe may be a useful site for studying the patterns and roles of synaptic plasticity. Correlative discharge signals in the lobe are associated with the electric organ discharge (EOD) motor command and are modified by pairing the command with sensory stimuli. The purpose of this study was to determine how this plasticity takes place in the lobe.

Single cells were recorded intracellularly in the ampylunar region of the electro sensory lobe. Intracellularly injected current pulses (instead of sensory stimuli) were paired with the EOD command. Clear changes in the synaptic effect of the corollary discharge were observed following pairing with depolarizing current pulses. Such pulses evoked both short (1 msec) and long (15 msec) duration spikes. The short duration spikes are presumed to be axonally or somatic and the long duration spikes are presumed to be dendritic. The long duration spikes appeared to be critical for plastic change but the short duration spikes did not. Only the short duration spikes are probably propagated down the axon to other cells.

Thus, modifications in the synaptic effect of the corollary discharge appear to result from pairing with post synaptic events that are restricted to the cells of the electro sensory lobe. Supported by NSF Grant BNS-8919627.

415.15

RELATIONSHIPS BETWEEN CARDIAC RESPONSE VARIABLES, URINARY CATECHOLAMINES AND MEASURED INTELLIGENCE IN EIGHT-YEAR-OLD CHILDREN. C.J. McCallister, N.S. Jiang, N. H. McAdur, W. K. Nath*, and C. Reynolds*. Dept. of Educational Psychology and Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

As part of a larger study, 24 eight-year-old subjects (12 males, 12 females) were administered a standardized intelligence test, the Kaufman Assessment Battery for Children (K-ABC). Height, weight, blood pressure and heart rate were measured immediately before testing. Catecholamines were measured by high pressure liquid chromatography in one 24-hr unspun sample at least two days before or after the testing and these data were normalized using creatinine values. Diastolic (DBP) and systolic blood pressure (SBP) and heart rate (HR) were all significantly different by gender, as were norepinephrine and epinephrine. Dopamine, age, IQ, height and weight were not significantly different by gender although weight approached significance. Correlations between SBP and IQ were significant for boys and girls were negative (r= -0.60, p<0.03, r=-0.59, p=0.04 respectively). DBP correlated negatively with IQ for boys but not for girls (r= -0.40, p>0.06). No significant correlations were found between any urinary catecholamines in the 24-hr unspun sample and IQ although epinephrine exhibited a trend toward significance for girls only. No significant correlations were found between any urinary catecholamines in the 24-hr unspun sample and cardiac response variables although a trend toward significance for girls only. Exploratory data analyses using stepwise regression revealed that blood pressure was the best predictor of test performance. Further regression analyses will be utilized following the completion of the larger sample (n=100).

415.16


Individuals can learn to modify the amplitude of the 9-12 Hz mu rhythm in the EEG recorded over primary sensorimotor cortex in order to move a cursor to a target located at the top or bottom of a video screen (Wolpaw et al., Electroenceph Clin Neurophysiol 78:252-259, 1991). We are now exploring development of two-dimensional control.

Bipolar EEG is recorded from the scalp over central sulcus of each hemisphere. Right and left mu rhythm amplitudes are assessed 5 times/sec by fast Fourier transform. A cursor appears in the middle of the screen and target appears in one corner. The cursor moves in two dimensions as a function of the right and left mu rhythm amplitudes. The individual's task is to move the cursor to the target.

Initial results suggest that individuals can develop two-dimensional control and use it to reach targets with greater accuracy than accounted for by one-dimensional control. An EEG-based brain-computer interface may provide a significant new communication and control channel for severely disabled individuals.

(Supported by IBM Corporation and the New York State Science and Technology Foundation.)
415.17
A COMPUTER-BASED ANALYSIS OF PAVLOVIAN BEHAVIOR USING DORSAL MIDBRAIN STIMULATION AS THE UNCONDITIONAL STIMULUS. M.G. LAURAND,* R.C. GONZALEZ,* and E. THOMAS, DEPTS. OF BIOLOGY & PSYCHOLOGY, BRYN MAWR COLLEGE, BRYN MAWR, PA 19010

We have developed a method for the direct quantification of conditioned responding to an LED cerebellar camera reports the position of an LED above the animal’s head every 0.1 s. Movement, defined as a change in LED position, was determined using pre-CS, CS, US, and post-CS periods in a light/dark discrimination paradigm, using Sprague-Dawley rats. “Freezing” was defined as less movement during the CS than during the pre-CS period. Electrical stimulation (EBS) of the dorsal midbrain (DM) was the US. The UR was movement; jumping forward or sideways, circling.

Animals consistently froze on CS+ trials and were active on CS− trials. The difference between CS+ and CS− periods was significant. The difference between pre-CS activity and activity during the CS was significant from 0. These results, together with a measure of EBS efficacy in a conflict paradigm, suggest that EBS is an effective stimulus for aversive Pavlovian conditioning. The method will be expanded within a second camera to quantify the different qualitative aspects of conditioned responding currently assessed by judgement using videotapes.

416.1
NEUROLEPTICS BLOCK HIGH DOSE, BUT NOT LOW DOSE, HEROIN CONDITIONED PLACE PREFERENCES. K. Nader, A. Bochara, F. Harrington* and D. van der Kooy, Dept. of Anatomy, University of Tennessee, Memphis, TN 38118.

We hypothesize that two separate motivational systems in the brain underlie the rewarding effects of opiates, one that functions in drug naive rats and the other that dominates in drug dependent animals. These two systems can be double dissociated by lesions of the brainstem segmental pedunculopontine nucleus (which block conditioned place preferences for environments paired with opiates in drug naive rats) and by neuroleptic induced dopamine blockade (which abolishes conditioned place preferences for opiates only in drug dependent animals). Recently, we found that the neuroleptic pinematine (0.5 mg/kg i.p.) blocked the conditioned place preferences produced by 500 µg/kg heroin in naive rats, but had no effect on similar preferences produced by a 500 µg/kg (s.c.) dose of heroine. Are four place conditioning trials with 500 µg/kg sufficient to produce a dependent motivational state? We independently tested for dependence induced motivational effects of withdrawal in rats receiving only four 500 µg/kg heroin injections. Rats received four exposures to each of the two place conditioning environments, an environment which was paired with the absence of opiates 23 hours after the animals' most recent heroin injection. When tested in a drug free state by providing free access to both environments, dependent rats' show an avoidance of the withdrawal paired environment while naive rats show no significant conditioned preference or aversion to the environment paired with the absence of opiates. Rats given four 500 µg/kg heroin injections showed significant avoidance of the environment paired with the absence of heroin, however no such avoidance was seen in rats given four 500 µg/kg heroin injections or one 500 µg/kg injection. We conclude that after four 500 µg/kg heroin injections animals are in a dependent state and therefore, as our model predicts, their preferences are subject to neuroleptic blockade. Animals trained with one 500 µg/kg or four 500 µg/kg heroin doses are not dependent and their preferences were therefore immune to neuroleptic challenge. These findings further support a two system model of the motivational properties of opiates.

416.2

Morphine produces rewarding effects (as assessed by place conditioning) in drug naive animals, but is blocked by bilateral lesions of the segmental pedunculopontine nucleus (TPP) of the brainstem, but not dopamine blockade of the mesolimbic dopaminergic system. These results suggest that aversion produced by the high-reward condition had higher break points at the 0.04 mg/kg dose followed by a steep monotonic decrease in break point as haloperidol dose increased. Prior salivation for dependence induced motivational effects of withdrawal in rats receiving only four 500 µg/kg heroin in naive rats, but had no effect on similar preferences produced by a 500 µg/kg (s.c.) dose of heroine. Are four place conditioning trials with 500 µg/kg sufficient to produce a dependent motivational state? We independently tested for dependence induced motivational effects of withdrawal in rats receiving only four 500 µg/kg heroin injections. Rats received four exposures to each of the two place conditioning environments, an environment which was paired with the absence of opiates 23 hours after the animals' most recent heroin injection. When tested in a drug free state by providing free access to both environments, dependent rats' show an avoidance of the withdrawal paired environment while naive rats show no significant conditioned preference or aversion to the environment paired with the absence of opiates. Rats given four 500 µg/kg heroin injections showed significant avoidance of the environment paired with the absence of heroin, however no such avoidance was seen in rats given four 500 µg/kg heroin injections or one 500 µg/kg injection. We conclude that after four 500 µg/kg heroin injections animals are in a dependent state and therefore, as our model predicts, their preferences are subject to neuroleptic blockade. Animals trained with one 500 µg/kg or four 500 µg/kg heroin doses are not dependent and their preferences were therefore immune to neuroleptic challenge. These findings further support a two system model of the motivational properties of opiates.

416.3
HALOPERIDOL, QUININE ADULTERATION, AND EXTINCTION PRODUCE ELEVATIONS IN THE FORCE EXERTED DURING OPERANT RESPONDING BY RATS. R. T. D. Hammond, P. Bankin*, & A. Eifenberg

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Previous studies have shown that animals responding in a food-rewarded paradigm exert increased force in the presence of neuroleptic drugs. In the neuroleptic-induced elevation in emitted force a result of reward attenuation or, as others have suggested, a motoric-postural phenomenon? In this study, 3 groups of rats were trained to press a force-sensing operandum for 2.5 sec access to sweetened condensed milk. Testing consisted of 2 min sessions separated by a 50 min intertrial interval. When animals responded during the second session under conditions of 0.075 mg/kg haloperidol, no reward, or quinine-adulterated (0.025%) reinfomr, all experimental animals emitted significantly greater levels of peak force than controls. These preliminary results suggest that neuroleptic-induced increases in peak force may reflect reductions in the rewarding quality of food reinforcement.
416.5
IBOTENIC ACID LESION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS PRODUCES OVERWEIGHT AND MODULATES THE PREFERENCE THRESHOLDS FOR SACCHARIN.

In previous studies we showed that bilateral ibotenic acid (IBO) lesion of the lateral hypothalamic nucleus (LH) in rats results in an increase of gustatory preference. Recent data obtained for saccharin, with intra-lesional injection of saline or pimozide, with body weight and water intake deficits.

In the present experiment, we studied the effects of IBO on the perinatal hypothalamic paraventricular nucleus (PVH), which is known to produce certain changes (namely hyperphagia and obesity) opposite to those produced by LH lesion.

416.6
LESIONS OF LATERAL HYPOTHALAMIC NEURONES ALTER THE EFFECTS OF MORPHINE INJECTED INTO THE PARABRACHIAL AREA ON PREFERENCE FOR SACCHARIN.
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In previous studies, we showed that ibotenic acid lesion of the lateral hypothalamic neurons (LH) alters the effects of peripherally-administered morphine on rats preference for saccharin (Touret et al., Pharmacol. Biochem. Behav., 38, 385, 1990). Furthermore, we identified the gustatory relay, the parabrachial area (PBA) as one of the central sites of the effect of morphine (Atak et al. Soc. Neurosci. Abstr. 16, 600, 1990).

In the present study we sought to determine whether lesions of the LH could also modulate the effects of morphine injected into the PBA. During the preference tests, lesioned and sham lesioned rats were placed either on ad libium or restricted access to water. Increasing doses of morphine (50 ng to 800 ng) were bilaterally injected into the PBA 15 min before the preference test.

In parallel with our initial studies, we observed that (1) morphine injected into the PBA increased or decreased saccharin preference depending on the expected concentration; (2) lesioned rats exhibited altered preferred response in the presence of morphine. Furthermore, the effects of the LH lesion were stronger when rats were placed on restricted access to water. (Supported by INSERM grant 89-6.016 and FRMF).

416.7
EXCITOTOXIC LESIONS OF THE RAT LATERAL HYPOTHALAMUS ENHANCE ACQUISITION OF SCHEDULE-INDUCED POLYDIPSIA.

Schedule-induced polydipsia (SIP) is seen when hungry animals are exposed to the intermittent presentation of food. Food deprivation elicits the LH animals SIP. In the present experiment, the ability of rats bearing fiber-sparing NM DA lesions of the LH to acquire SIP was examined. Following surgery, 11 rats lesioned with 1.00-0.06M NM DA lost weight and were hypophagic and hypodipsic compared to 6 sham lesioned rats. None of the lesioned rats required wet mash or intragastric intubation. LH-lesioned rats showed an impairment in drinking over the course of the experiment but this was not statistically significant. Through all the testing all rats were placed on a food restriction regime to reduce body weight to 85% of normal. At the end of the experiment, LH rats had a lower body weight than controls, but this was not statistically significant. SIP was examined in 60 min sessions every other day; one 45mg food pellet was delivered every 60 sec, while water was available ad lib via a borette protruding into the operant chamber. There were significant differences between the groups over the first 6 sessions (groups P(14)=5.19 p<0.05; interaction F(5,70)=2.34 p<0.05). LH lesioned rats acquiring SIP more quickly than controls. This data show that there is no motor impairment in LH lesioned rats; and that the LH may be involved in higher-order processes relating to the control of motivated behavior.

416.8
WATER DEPRIVED RATS REMAIN THIRSTY DURING DOPAMINE RECEPTOR BLOCKADE.
J.C. Horvitz* and A. Friedman*. Dept. of Psych., Univ. of Cali., Santa Barbara, CA 93106.

In order to determine whether dopamine receptor blockade could attenuate thirst, the drinking behavior of rats under 23, 16, 12, and 0 hr water-deprivation was compared to that of 23-hr deprived animals under the influence of either 0, 0.5, 0.75, or 1.0 mg/kg of pimozide. Reductions in levels of water-deprivation were produced a) increased latencies to initiate drinking, b) changes in the pattern of licking over the course of the session and c) decrements in the total number of licks during the session. In contrast, administration of pimozide to 23-hr deprived animals produced no effect on either a) initiation latencies or b) lick patterns, and c) only marginally reduced the total number of licks during the session. Further, individual lick durations and interlick intervals were not affected by the neuroleptic. These data suggest that neither-thirst mechanisms nor motoric processes underlying drinking behavior are disrupted by dopamine receptor blockade.

416.9
REINFORCING PROPERTIES OF DRINKING WATER: ROLE OF OPIOIDS AND DOPAMINE.
Veronica Navarro*, Irit Federman* and Anders Agmo. Dept. of Psychology, Universidad Anahuac, Mexico City.

We have previously reported that sucrose drinking in non-deprived rats produces conditioned place preference. Consumption of the sucrose solution was taken to represent reinforcing properties and the place preference was taken to be a direct indicator of this reinforcement. A dopamine antagonist blocked reinforcement but not reward, while an opiate antagonist blocked reward but no reinforcement.

The purpose of the present experiment was to evaluate whether water-deprived rats and the reinforcer produced by this event responded in the same way to dopamine and opioid agonists as sucrose drinking and reinforcement. Male rats were deprived of water for 24 hr and then allowed to drink for 8 min in a lickometer. Immediately thereafter they were placed in place preference cagemats. The non-preferred cage was the initially non-preferred cage. After a total of three reinforced and three non-reinforced sessions, the test was made. Drinking produced a place preference that was blocked by pimozide, 1 mg/kg, administered before the reinforced sessions. A slight reduction of drinking was also observed. It is possible that the reinforcer produced during reinforced sessions produced place aversion and considerable and progressive reduction of drinking. These data suggest that dopamine affects reinforcement and opioids reward.

416.10
SCopolamine BLOCKADE OF AMPHETAMINE-INDUCED CPP

Neurochemical and behavioral investigations support the existence of a DA-ACH reciprocal interaction in the neostriatum, which is apparent from opposite changes in turnover and in behavioral consequences of pharmacological challenge. While data have been reported implicating ACh in DA-mediated behaviors from dopamine (dopamine) and ventral (hyperactivity) striatum, exact nature of the DA-ACH reciprocal interaction is not known: reports can be cited suggesting either a reciprocal relationship or no interaction. Amphetamine-CPP arises from DA activation in the (striatal) NAS region. DA in this structure has been repeatedly implicated in reciprocal interaction processes. In the present study, 4 groups of (n=8) rats were conditioned with 1.5 mg/kg of Ap via the nonpreferred side of a CPP chamber. A 30% significant increase in time spent on this side over control (6.33 to 26.8% over 15 min). Conditioning with 0.1 mg/kg scopolamine co-administration blocked the increase, although failing to induce a significant aversion itself. Locomotor activity data suggested that Sc also blocked conditioned hyperactivity but the magnitude of these changes failed to reach significance. These findings implicate a cholinergic component in the limbic circuitry generating Aap-CPP, and suggest the possibility that this component opposes DA as with striatally-mediated behavior.
417.1 AUDITORY FEEDBACK MAINTAINS CRYSTALLIZED SONG IN ADULT ZEBRA FINCHES. E.K. Nordeen, and R.D. Nordeen. Dept. Psych., Rockefeller University, Millbrook, NY 12545

Birds use auditory feedback in learning song, but differ in their use of this feedback in maintaining adult song. Earlier work suggested that birds that learn song during critical periods, eventually attain a central motor program as adult song patterns "crystallize". However, we have found that the crystallized songs of zebra finches change little from day to day, and that vocalizations do not follow the pattern of long-term adult song. We recorded the songs of 25 adult (>120d) male zebra finches and then removed the cochleae from 11 of these birds. Songs were recorded up to 16 weeks following surgery (deaf) or after 15-6 weeks (control). If by 16 weeks after surgery deaf birds accurately reproduced only 36% of the songs they previously recorded, in contrast, control birds retained 90% of their syllables. Also, the percentage of syllables that were unmatched or only slightly similar in phonology to previously recorded syllables was higher in deaf birds than in controls. Usually, these effects of deafening took 6-8 weeks to develop. We do not yet know where auditory feedback acts to maintain the neural circuits supporting adult song behavior, but preliminary data suggest that lesioning the lHMX, which directs song development, does not compromise the long-term maintenance of adult song.

417.3 SONG INDUCES "IMMEDIATE EARLY" GENE EXPRESSION IN SONGBIRD FOREBRAIN. C. Mello*, D. S. Vicario and D. F. Clayton. Lab of Animal Behavior, The Rockefeller University, NY 10021, & Beckman Institute, University of Illinois, Urbana, IL 61801

We identified a gene induced in songbird associative forebrain in response to the sound of birdsong. This gene ("ZENK") encodes the canary homolog of an "immediate early" transcription factor known to be induced in neurons for neural growth and degeneration (Mello & Clayton, Soc Neurosci Abstr 16:657, 1990). By in situ hybridization, ZENK mRNA levels increased ~10-fold in the forebrain of canaries or zebra finches that heard tape-recorded birdsong after 1 day of acoustic isolation. The most marked induction occurred in the medial caudal neostriatum (NCM) when birds were exposed to song of their own species. Little or no induction occurred in response to pure auditory tones, or in the defined song control circuit or Field L. NCM may represent a site for: 1) auditory discrimination; 2) song storage; or 3) multimodal associations involving the song stimulus.

417.5 A TEST OF THE AERODYNAMIC WHISTLE HYPOTHESIS FOR THE PRODUCTION OF BIRDSONG. R.A. Suthers and M.X. Zuo*. Medical Sciences, Indiana Univ., Bloomington, IN 47405

Birdsong is often assumed to be generated by the vibration of tympaniform membranes in the avian vocal器官---the syrinx. However, an alternate mechanism of sound production based on the principle of an aerodynamic whistle, has also been hypothesized to generate certain vocalizations by avian species. Studies have included the zebra finch (Columbaeformes) (Gauts and Gauts & Casey, Auk 99:474, 1982). According to the whistle hypothesis, sound is produced by a series of stable vortices in the respiratory airflow from the syrinx. These vortices are produced in laminar airflow downstream from the syrinx and eliminate any turbulent vortices in the trachea. Vocalizations in which the syrinx functions as an aerodynamic whistle should be eliminated by this "flow straightener" whereas sounds produced by vibrating membranes should be little affected. In a series of experiments, airflow through the trachea and through the mouth and nostrils was measured using heated microhotwire. Insertion of the tracheal flow straightener did not significantly reduce air flow velocity, indicating vocalizations produced by male pigeons when presented with a female. This result suggests that cooing is not produced by an aerodynamic whistle in the syrinx. (NSF BNS 8720192)

417.2 BRIEF EXPOSURE SONG LEARNING IN ZEBRA FINCHES: ACQUISITION AND PRODUCTION OF A MODEL WITH ONE DAY OF TUTORING. A. Lombardo and P. Lenzo. Dept. of Biology, Hunter College, New York City, NY 10031

Juvenile male zebra finches, Taeniopygia guttata, normally learn the complex vocalizations of courtship song from an adult conspecific male with whom they can interact. Song learning in this species consists of two overlapping phases: an early sensitive period for acquisition of a model, and an extended period of vocal development during which the model is imitated. It is not known what minimal amount of song exposure is sufficient for imitation to occur. Here the auditory and social environments of juvenile finches were manipulated from one week after hatching until sexual maturity (day 90) in an attempt to narrow the period of model acquisition. Juvenile finches were flown from their mothers until day 28; on that day each bird was placed singly with an unrelated adult female. On day 35 an adult male ("tutor") was placed in the scholar's cage for 24 hours. The scholars had no previous contact with a male until day 90. Six control juveniles were treated in the same manner but had no access to a tutor male. The process of song development in both groups was followed by tape recordings and vocalizations at days 45, 60, 75, and 90. Visual inspection of sonograms of each juvenile's song and his respective tutor revealed moderate to marked imitations of the model in four of the six scholars, whereas controls produced none of the complex syllables found in the tutors' songs, often incorporating the female companion's calls into a male song structure. The song of scholars and controls had similar introductory notes, which probably are not learned from male tutors. Experimentally restricting the time frame in which zebra finches acquire their song should greatly facilitate study of the mechanisms of song learning.

417.4 DEVELOPMENT OF AUDITORY RESPONSES IN NUCLEUS HVC WITH REDUCED AUDITORY FEEDBACK DURING SONG ACQUISITION. S.E. Volman. Dep. of Zoology, The Ohio State U, Columbus OH 43210

The sensorimotor stage of song learning in white-crowned sparrows lasts for about 1 month. During this period, birds match their own "plastic" song to a stored memory of a song model. Before this stage, auditory neurons in the song-control nucleus HVC show little evidence of selectivity for the song model, and auditory responses habituate easily. During motor song, auditory neurons become more responsive to song in general, and also become preferentially responsive to a bird's own song (Volman and Konishi, Soc. Neurosci Abst., 1987). This study asked if auditory responsiveness was necessary to produce these changes in the auditory responses of HVC, or might they, at least in part, be a consequence of experience with the song model? Neurons were recorded from the HVC of four juvenile sparrows that were isolated from the auditory and social environments of male zebra finches were manipulating from 1 day of acoustic isolation, and were fed for at least 30 days. Desynchronization was accompanied by partial opening of the interauricular air sac, which surrounds the syrinx. The air sac heals in 5-7 days, so vocalizations were monitored every day to determine when the birds were imitating their models. Each bird was able to hear himself sing for a total of about 4 days during this time. The responses of HVC neurons, recorded after the 30+ days of devocalization, were compared on a variety of measures to those in normal birds at an equivalent time after vocalization begins. The HVC neurons in devocalized birds showed robust responses to song and showed some song selectivity, but this was markedly less than that in normal birds. These results suggest that auditory responsiveness may develop in HVC in the absence of auditory feedback, but the mature pattern of song-selectivity requires such feedback, and does not simply reflect the activation of response patterns established during the acquisition of the song model.

Supported by NIH and the Deafness Research Foundation

417.6 INTRINSIC OSCILLATORY PROPERTIES OF VOCAL PREMOTOR NEURONS IN THE ZEBRA FINCH FOREBRAIN. B. Mooney. Div. of Biology 216-76 Caltech, Pasadena, CA 91125

The zebra finch brain contains several nuclei that are specialized for vocal control during singing. Nucleus RA, located in the caudal forebrain, contains neurons that project directly onto the motoneurons controlling the syringeal muscles used in song. To learn how these neurons control vocal motor control, their intrinsic electrophysiologic properties were studied in an in vitro slice preparation (Columbaeformes) (Gaunt and Gaunt & Casey, Auk 99:474, 1982). According to the whistle hypothesis, sound is produced by a series of stable vortices in the respiratory airflow from the syrinx. These vortices are produced in laminar airflow downstream from the syrinx and eliminate any turbulent vortices in the trachea. Vocalizations in which the syrinx functions as an aerodynamic whistle should be eliminated by this "flow straightener" whereas sounds produced by vibrating membranes should be little affected. In a series of experiments, airflow through the trachea and through the mouth and nostrils was measured using heated microhotwire. Insertion of the tracheal flow straightener did not significantly reduce air flow velocity, indicating vocalizations produced by male pigeons when presented with a female. This result suggests that cooing is not produced by an aerodynamic whistle in the syrinx. (NSF BNS 8720192)

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417.7

NONLINEAR RESPONSE PROPERTIES OF AUDITORY NEURONS IN THE ZEBRA FINCH'S SONG SYSTEM: A QUANTITATIVE ANALYSIS

D. Lim, A. J. Doupe, and M. Konishi. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Auditory forebrain song nuclei of the male zebra finch (Poephila guttata) contain neurons sensitive to the bird's own song. These neurons respond best or exclusively to a certain configuration of song acoustic elements (E. S. Fortune, D. Margoliash, 1989 Adv. Soc. Neurosci). The response of these neurons to the effective configuration does not appear to be a simple addition of their responses to individual elements presented in isolation, indicating the involvement of non-linear processes. To quantify these nonlinear interactions, we used a matrix-based optimization method (D. Lim and R. R. Caporale, 1990 J. Acoust. Soc. Am.). The response of the neurons to the effective configuration does not appear to be a simple addition of their responses to individual elements presented in isolation, indicating the involvement of non-linear processes. To quantify these nonlinear interactions, we used a matrix-based optimization method (D. Lim and R. R. Caporale, 1990 J. Acoust. Soc. Am.).

For example, we studied responses of single HVC neurons to harmonically related tones and their combinations such as f1, f2, f1+f2, f1+f3, f1+f2+f3 etc. The results showed different dependencies. For example, we studied responses of single HVC neurons to harmonically related tones and their combinations such as f1, f2, f1+f2, f1+f3, f1+f2+f3 etc. The results showed different dependencies. Further, it lent itself well to generating the set of most meaningful acoustic components for a particular group of neurons. Thus, the selectivity of forebrain nuclei for a given set of stimuli could be represented in terms of their nonlinear dependencies. Further, it lent itself well to generating the set of most meaningful acoustic components for a particular group of neurons. Thus, the selectivity of forebrain nuclei for a given set of stimuli could be represented in terms of their nonlinear dependencies. For example, we studied responses of single HVC neurons to the bird's own song.

Discrimination. Again, there were no differences in song discrimination. Again, there were no differences in song discrimination. The following study was to determine whether the song control nuclei of house sparrow were dependent upon the song control nuclei of house sparrows. This is the First report that song discrimination is lateralized. Seventeen adult male zebra finches were divided into seven pairs. In each pair, a bird was given a right- or left-side lesion of the telencephalic song control circuitry. This operation did not affect song production, but it affected performance in song discrimination tasks. Left-lesioned birds in each pair required more trials to reach a learning criterion than right-lesioned birds. However, when the same birds were asked to discriminate between songs varying only in the placement of one frequency component in a harmonic structure, left-lesioned birds in each pair mastered the task much faster.

This suggests that the hemispheres of the songbird brain may process song signals in different ways, paralleling inter-hemispheric perceptual differences reported in humans and other primates.

Effects of early sensory experience on neural and behavioral plasticity have been linked to activation of N-methyl-D-aspartate (NMDA) receptors. Early auditory experience appears to be critical for normal song learning in zebra finches, and the physiology of one song control area, the robust nucleus of the archistriatum (RA), indicates the presence of NMDA receptors in young males (Mooney and Konishi, ’89). To further localize NMDA receptors in the song system, we measured glycine/glutamate-enhanced binding of 3H-MK801, an NMDA receptor antagonist, in brain sections from adult (>120 day) male zebra finches using film autoradiography. The MK801 binding was quite low in RA, the higher vocal center (HV), and the lateral magnocellular nucleus of the anterior neostriatum (IMAN). In fact, binding within all of these regions was distinctly less than in adjacent tissue. In contrast, specific MK801 binding was high throughout the parolfactory lobe, including Area X, a region critical for song learning. We are currently examining MK801 binding in 30 day and 50 day old juveniles to determine whether NMDA receptor densities in these song related nuclei are higher during the sensitive period for song learning than in adulthood.


In songbirds estrogen (E) is important for the growth of forebrain song nuclei, juvenile song acquisition, and the activation of adult song behavior. (Gurney 1981; Harding et al. ’83; Norden & Noedeen ’89; Marler et al. ’87).

The accumulation of sex steroids in cells in song nuclei is thought to mediate hormonal effects. Gah et al. (1987) showed that cells in the caudal nucleus of the ventral hyperstriatum (HVc) of male canaries have E receptors (ERs).

We asked if female canaries have E receptors (ERs). E receptors can bind androgen, but do so only rarely and more simply than males, they provide a test of the hypothesis that the ability of both sexes of a species to song require E accumulation by HVc cells has yet to be determined.

The brains of 4 male and 4 female canaries in breeding condition were cryosectioned at 16-25 µm. Sections were incubated with the H222 monoclonal antibody raised against ERs (Abbott Labs, North Chicago, IL) against the ABC method, and visualized with DAB.

Cells immunoreactive with the ER antibody were observed throughout HVc in both sexes. The proportion of ER (+) cells in HVc did not differ between females (22.4 ± 5.9%) and males (20.8 ± 5.9%). The absolute number of ER (+) cells in HVc was greater in females (236.6 ± 4 µm²) than males (31.1 ± 7.5 µm²).

These results support the hypothesis that the presence of ERs in HVc cells of both sexes is a necessary preadaptation for bisexual song. HVc also acts in song perception as well as song production (Brenowitz ’91).

Therefore, sex steroids may influence the activity of neurons important for both the production and perception of song. (Supported by NIH DC487, Sloan Fellowship to EB, Howard Hughes Predoctoral Fellowship to GTS).


The number of neurons in vocal-control circuits of adult canary brain varies as a function of season and circulating levels of sex steroids. Although the rate of incorporation of newly generated neurons into vocal-control circuits varies seasonally, this difference could reflect a variation in neurogenesis, a lower rate of cell death, or an altered migration. We are interested in the factors that control the rate of cell proliferation, and have looked for changes in the incidence of thymidine-labeled cells in the forebrain ventricular zone of adult canaries as a function of variations in hormone levels.

Adult female canaries were anesthetized and gonadectomized. Four separate groups of birds received systemic exposure to either testosterone, estradiol, a combination of an anti-androgen (flutamide) and an aromatase inhibitor (ATD), or nothing. All birds were also implanted with an osmotic mini-pump that released 1H-thymidine at a rate of 1 µL/h (g, 1.5 uCi/g/day) for three days and were killed four days following onset of treatment. Analysis of autoradiograms revealed no differences between groups in the incidence of labeling within the ventricular zone from forebrain levels around the anterior commissure. Preliminary analysis of similarly treated birds allowed us to survive 7 days following onset of treatment yielded comparable results. These results suggest that sex steroids do not directly regulate the rate of cell division in the ventricular zone. Differences in the incorporation of labeled cells into vocal-control nuclei may therefore be due to regulation of neurogenesis by other factors or to some other cellular mechanism.


Male canaries modify their songs seasonally, adding new and losing old syllables at a time when serum testosterone (T) levels are low (Notebohm et al. ’82). Evidence that seasonal T levels vary seasonally and correlate positively with seasonal variation in the Nist-defined volume of HVc (a brain region necessary for song production), suggests that T may regulate the size of specific cell populations in HVc (Notebohm ’81). However, at least two populations of HVc cells show no seasonal variation in their distribution: estrogen target cells and neurons which project to Area X (Gahr ’90). We have measured the incidence of estrogen target cells in HVc and asked whether the distribution and/or number of these cells could be influenced by exposure to T.

Adult males were maintained on short days, gonadectomized, and treated systemically with either T or a combination of an anti-androgen (flutamide, FL) and an aromatase inhibitor (ATD). One month later birds were processed for 3H-dihydrotestosterone (DHT) autoradiography. T treatment increased both the Nist-defined volume of HVc and the proportion of DHT target cells in HVc. Thus, FL/ATD birds have a smaller HVc with many fewer DHT target cells. Moreover, in contrast to estrogen target cells, the distribution of DHT target cells matched precisely the Nist-defined borders of HVc in both T- and FL/ATD-treated males. These data suggest that T can regulate the size of a population of estrogen target cells in HVc, and in this way may influence seasonal relearning and modification of song.


Vocal behavior in songbirds is controlled by a system of interconnected brain nuclei. Previous studies indicate that discrete portions of this neural system are critical for learning, but are not needed for maintenance of song. Lesions of either IMan or Area X in juvenile birds disrupt vocal performance whereas ablations in adults do not disturb the stable song pattern. Area X projects trans-synaptically to IMan through the thalamic nucleus DLM, which appears to provide the sole source of afferent input to IMan in adult canaries. In order to examine the effect of lesions at different levels in the X-DLM-IMan circuit, DLM afferents to IMan were ablated by knife-cut in young males before 50 days of age and in adult males between 80 and 100 days old. Damaging only DLM afferents disrupts the normal vocal pattern in juvenile males but has no effect on the stereotyped song of adults, as is true for knife-cut in young males. However, the nature of the song deficits appears to vary in relation to the level of interruption in the X-DLM-IMan circuit. Zebra finches receiving lesions of IMan at a young age show only a few grossly abnormal notes. In contrast, severing the DLM input to IMan in juvenile males renders a poorly modulated song consisting of an unstable sequence of 3 to 5 notes. These findings suggest that removal of the input to IMan is less disruptive to the development of vocal behavior than are lesions to IMan itself.

This difference in the nature of the behavioral deficits may reflect the neural processing within each portion of the X-DLM-IMan circuit.


The song system can be divided into two subsystems: a caudal group of nuclei that has a large role in crystallized song production, and a rostral group that has a large role in song acquisition. Song acquisition typically consists of an early phase of learning and a later phase of auditory-motor integration. In development, major changes in the morphology of the rostral system occur during the initial auditory phase, suggesting that these regions play a role in song acquisition.

We now find that one rostral nucleus, I-Man is also needed for the later phase. Adult female canaries were implanted with testosterone (T). This makes them produce a stereotyped song like that produced by adult males (but with a repertoire of c. 8 syllables rather than 20-30). After the birds were singing, we lesioned I-Man bilaterally. In a second set of females, I-Man was lesioned first and T was then given. Vocalizations of both groups were analyzed. In birds that were already singing, the lesions did not appear to alter the number of syllables in the repertoire, the stereotypy of syllable production or the patterning of syllables (although a few syllables appeared modified after the lesion). In contrast, birds that received the lesion before T, put together a song with only 2-3 simple syllables.

These data suggest that I-Man plays a role in the auditory-motor phase of song acquisition, even when song is acquired as an adult. As such, this lesion may be useful for studying female song. Thus auditory-motor integration can be induced and studied in adult animals, perhaps making it possible to determine neural changes responsible for this aspect of song learning. Supported by HD21035-05.
417.19

DOPAMINE RECEPTORS IN THE SONG CONTROL SYSTEM: AREA X IS DEFINED BY THE D2 BUT NOT THE D1 DOPAMINE RECEPTOR SUBTYPE. J. M. Card, G. F. Ball and J. Ballagh.

Dept. of Psychology, Bates College, Chestnut Hill, MA 02167.

The songbirds possess a system of sexually dimorphic brain nuclei that mediate the production and acquisition of complex vocalizations (i.e. song). One of these nuclei, area X, is a subdivision of the parolfactory lobe (PO), the caudate-accumbens nucleus. Area X is marked by somatotopic and is known to receive a dopaminergic projection from the area ventralis of Tsai (AVT) (Lewis et al., 1987). To better understand this dopaminergic system, the activity in area X and LPO was mapped by quantitative autoradiography. The pattern of NMDA receptor binding is different in Area X and LPO. The binding of NMDA receptors was defined by a high density of D2 receptors as compared to the surrounding LPO. In the case of the D1 subtype there was no apparent difference between area X and the surrounding LPO. In the songbird species, Area X, in the LPO, was clearly defined by a high density of D2 receptors as compared to the surrounding LPO. In the case of the D1 subtype there was no apparent difference between area X and the surrounding LPO.

417.20


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The N-methyl-D-aspartate (NMDA) receptor type has been implicated in the control of learning and memory in several vertebrate species. Songbirds possess a set of interconnected brain nuclei that are involved in the acquisition and modification of vocal behaviors. To assess the possible involvement of this receptor subtype in song, we mapped, by in situ quantitative autoradiography, the patterns of NMDA receptor binding in the songbird brain. The non-competitive antagonist [3H] MK-801 was used to label the receptors. An increase in the specific binding (as defined by competition with ketamine to define non-specific binding). Two songbird species, the zebra finch (Taeniopygia guttata) and the European starling (Sturnus vulgaris), were studied. These results suggest that the NMDA receptors play a role in the control of learning and memory in these birds.

418.1

NEURAL CORRELATES OF EXPLORATORY BEHAVIORS IN FREELY-MOVING NAVAJAN INERMIS (MOLLUSCA; OPISTHOBRANCHIA). J. L. Leonard, Mark O. Hatfield Marine Science Center, Oregon St. Univ., Newport, OR 97366.

Navanax is a predatory hemipranchid whose normal sexual behavior involves bouts of copulation in which a pair of individuals repeatedly alternate sexual roles. In an attempt to elucidate the role of the CNS in these long-term behaviors, I have used 2-14 h observation periods on 2-4 consecutive days to record repeated instances of whole behavioral sequences from animals with chronically implanted bipolar cuff electrodes. Use of a split-screen technique makes it possible to record, visualize, and play back CNS activity on intact nerves or commissures, simultaneously with behavioral data. The high degree of cryp tic neural activity and the fact that Navanax behavioral states are not mutually exclusive, make it difficult to identify one-to-one correspondences between behaviors and neural patterns. Also, individuals with lesions of major nerves often show few behavioral deficits. However, recordings from the 5th pedal nerve show a pattern of low frequency bursts of medium amplitude (~0.3-0.5 mV) which appears to be associated with swimming (presumably chemosensory) of the substrate. It may be related to flaring of the Hancock's organ. These spikes are normally absent during contact with prey and may therefore be associated with recognition of, or searching for, conspecifics. There is little overlap between spikes making it possible to identify single units. This suggests that it is possible to identify the neuron responsible for these spikes. More variable patterns of activity on P5 are associated with head-turning. Supported by NIH and NSF.

418.2


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Mechanical and chemical stimuli elicit defensive acid secretion from the skin of P. californica (Gillette et al., J. Exp. Biol., 156, 335 (1991)). Skin acidification potentiates aversive withdrawal of the affected body part and potentiates aversive turns and locomotion. It was shown that stimuli that elicit acid secretion also suppress feeding behavior and raise feeding thresholds. We have now found that appetitive food stimuli also stimulate acid secretion at the oral veil, suggesting that acid secretion could interact with an animal's motivational state to regulate feeding behavior. To test this, skin acid secretion was recorded while appetitive stimuli (squirrel homogenate or trimmed/glycine) were applied to the oral veil of animals before and after satiation. Feeding thresholds rose by an average of 1.75 log units, but oral veil acidification (avg. 1.19 ± 1.09) was not significantly affected by satiation. These data suggest that food chemosensory stimulation of acid secretion interacts with satiation state to limit meal size. Differential chemosensory conditioning may help to further clarify the role of acid secretion in regulation of feeding behavior.

418.3


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P. californica learns to discriminate chemosensory stimuli such as squid homogenate, sea anemone homogenate, and beer extract (Davis et al., J. Comp. Physiol., 138, 157 (1980); Mptson and Cohan J. Neurobiol., 17, 487 (1986)). A simple computational model has been proposed to study possible mechanisms by which complex olfactory stimuli are encoded and discriminated. The model incorporates 1) broadly but distinctly tuned olfactory receptors, 2) simple contrast enhancement mechanisms, 3) documented interactions of neural circuitry for appetitive and aversive behavior, and 4) simple learning mechanisms tied to nociceptive and reward pathways. Results so far suggest that expression of a repertory of relatively complex behavior may be appropriately and specifically altered by simple heterosynaptic modification of sensory paths to specific motor network elements.

418.4

LEARNING CORRELATED CHANGES IN A CALCIUM-ACTIVATED K CURRENT IN HERMISIENDA TYPE A CELLS. Y. Han and J. Farley.

Program in Neural Science, Indiana University, Bloomington, IN 47405.

Previous research has determined that Type A photoreceptors in the eyes of Hermisenda exhibit reduced light-induced depolarizing generator potentials and reduced responses for several days following pairings of light and rotation, when compared to results from random control or untrained specimens (Behav. Neuroscience, 1990). Voltage-clamp studies indicate that a T-type and 4-AP calcium-dependent potassium current (I_{cal}) is an important determinant of a Type A cell's light response. In the present study, generator potential amplitudes of sympathetically-isolated T-type cells from trained and control A specimens were measured in normal and calcium-free ASW on retention days following learning. The ~5.0 mV training-associated difference in generator potential amplitude between A cells from trained and control A specimens was virtually eliminated when calcium was removed from the bathing media. Measurements of I_{cal} failed to reveal any training-associated difference in this component of K current, while measurements of I_{cal} revealed greater current amplitude (~25% for potential difference ~25% for potential difference >35 mV) in A cells from trained vs. control specimens. Collectively, these results demonstrate that long-term training-associated differences in Type A cells' light responses are due in part to a training-induced increase in I_{cal}, a mechanism which is the converse of that which underlies learning-associated changes in Type B photoreceptors.
418.5  IDENTIFICATION OF A GABAB RECEPTOR THAT CONTRIBUTES TO VISUAL-VESTIBULAR INTERACTIONS IN HERMISSEND A L.D. Mattel and D.M. Bass  Department of Psychology, Rutgers University, New Brunswick, NJ 08903

Stimulation of vestibular hair cells induces a hyperpolarization and cessation of firing in ipsilateral B photoreceptors in the mollusk Hermisenda. This response is mediated by the function of which cannot be exclusively attributed to an inward Cl conductance at the B cell soma (e.g., Mattel & Alkon, Brain Research, 1991). At resting membrane potentials (50 to 60 mV), pretreatment with either GABA (125 μM) or the GABA antagonist baclofen (125 μM) to the terminal branches of the B cell induced a hyperpolarization analogous to that induced by haircell stimulation. though the hyperpolarization in baclofen was reversed relative to the hyperpolarizing response to GABA (4 ± mV and 8 ± mV, respectively). The GABA-induced hyperpolarization was reduced by pretreatment with baclofen: GABA, antagonistic bicuculline (300 μM) or the GABA antagonist 2-hydroxysaclofen (450 μM), but was eliminated by the combined application of both antagonists (150 μM and 225 μM, respectively). Moreover, the potential at which GABA-induced net voltage response shifted from -40 mV to -70 mV when either bicuculline (300 μM) or 2-hydroxysaclofen (450 μM) were added to the extracellular solution. To determine the conductances changes underlying these voltage responses, the B cell was voltage-clamped at -55 mV, and GABA application was found to induce a net inward current. Following application of 2-hydroxysaclofen (450 μM), the magnitude of the inward current increased, while application of bicuculline (300 μM) revealed an outward current in response to GABA, consistent with the persistence of an outward K+ flux induced via stimulation of a GABA receptor. In total, these results indicate that the voltage response of the B photoreceptors to GABA is the sum of an inward and an outward current, which arise from dual activation of both GABAA and GABAB receptor subtypes.

418.6  CORRELATES OF CONDITIONING IN PAIRS OF IDENTIFIED TYPE A & B PHOTORECEPTORS IN HERMISSEND A: EFFECTS OF ADAPTATION, LIGHT INTENSITY AND SYNAPTIC INTERACTIONS L. J. Flores*. Molecular Biophysics Research Department, AT&T Bell Laboratories, Murray Hill, NJ 07974

In response to a mechanical tap the nematode Caenorhabditis elegans shows a withdrawal reflex, swimming backwards for some distance. This reflex is capable of habituation and sensitization, as well as both short and long term memory. In addition, we have discovered two antagonistic reflexes in C. elegans. Antagonistic reflexes that use the same final common path cannot be activated simultaneously, therefore, if we fed one group of snails ad lib, while a second group was starved for 5 days. Snays converging from the GCN were identified within the brain, after injecting the GCN with hexamminecobalt. The principal result was that the ratio of flat synapses to flat synapses was significantly greater in the starved animals than in the sated animals. There were no significant differences between the groups in regard to the numerical densities of labelled synapses, the vesicle numerical densities, or the synaptic strength. These results imply that sensory information about food is transmitted more effectively to the GCN in hungry snails than in sated snails.

418.7  EVALUATION OF MALE REPRODUCTIVE FUNCTION IN THE PULMONATE SNAIL MELampus EIGENIGHTUS AFTER PENIAL COMPLEX EXCISION AND NERVE AND GANGLION LESIONS S. B. Moffett. Department of Zoology, Washington State University, Pullman, WA 99164

The penial complex of Melampus is innervated by two clusters of neurons, a right cerebral cluster that is excitatory, and a right pedal cluster that is inhibitory (Moffett, Neurosci. Abs. 1989). Specific innervation of the penial complex occurs after the axons are severed (Ridgway et al., Neurosci. Abs. 1988) and the penial complex regenerates if at least part of its innervation is intact. The copulatory success in these hermaphroditic animals is tested in regard to the numerical densities of synapses contacting a molluscan feeding interneuron. R. Chase and B. Tolloczko. Cerebral Neuron (GCN), a molluscan serotonergic interneuron. Food deprivation increases the curvature of food is transmitted more effectively to the GCN and to facilitate feeding behavior. Snails with the same lesion history are maintained together and egg development is observed. Physiological and anatomical persistence of an outward K+ flux induced via stimulation of a GABAB receptor. In total, these results indicate that the voltage response of the B photoreceptors to GABA is the sum of an inward and an outward current, which arise from dual activation of both GABAA and GABAB receptor subtypes.

418.8  ODOR RESPONSES AND OUTPUTS OF AN OSCILLATING OLFACTORY PROCESSING NETWORK IN A TERRESTRIAL MOLLUSK, A. Gelperin and L. Elsässer. Molecular Biophysics Research Department, AT&T Bell Laboratories, Murray Hill, NJ. 07974

Odor processing networks in a variety of species are being explored from both cellular and computational perspective to determine how molecular recognition and categorization are accomplished and how the resulting information is interfaced to behavioral subsystems which require processed odor information, such as navigation and odor learning. The cerebropal (PC) lobe of the terrestrial slug Limax maximus is such an odor processing network. The PC lobe receives direct input from both olfactory and superior regions and displays coherent network oscillations at 0.7 Hz which are modulated in waveform and frequency by natural odor input (Nature 345:437, 1990). The natural odor dopamine (2-methoxyphenol; dopamine) produces comparable changes in the PC lobe oscillation when applied to the neuropil of the superior nerve in solution (10^-4 % in saline) or as the vapor phase of a 10^-5 % aqueous solution. Candidate outputs of the PC lobe were identified by application of the fluorescent carboxycyanine dye DiC1(3) to the neuropil of the PC lobe and incubation of the tissue at room temperature for 12 to 36 hours. A small number of labelled fibers (4 ± 2, N = 15) mean ± std.dev. appeared in the cerebropedal connective as did labelled cells in the pedal ganglion (8 ± 5, N = 13). The pedal cells appeared in 1 - 3 clusters. By analogy to similar cells in Achatina described by Chase and Tolloczko, the Limax pedal cells with processes in the PC lobe are likely to carry output from the PC to locomotor control centers in the pedal ganglion.

418.9  FOOD DEPRIVATION INCREASES THE CURVATURE OF SYNAPSES CONTACTING A MOLLUSCAN FEEDING INTERNEURON. R. Chase and B. Tolloczko. Department of Biology, McGill Univ., Montreal, Quebec, H3A 1B1 Canada

The purpose of this study was to investigate the plasticity of the synapses on the Giant Cerebral Neuron (GCN), a molluscan serotoninergic cell that responds to food stimuli and to facilitate feeding behavior. Snails (Lumina decollata) commonly experience extended periods of food deprivation. Therefore, we fed one group of snails ad lib, while a second group was starved for 5 days. Snays converging from the GCN were identified within the brain, after injecting the GCN with hexamminecobalt. The principal result was that the ratio of flat synapses to flat synapses was significantly greater in the starved animals than in the sated animals. There were no significant differences between the groups in regard to the numerical densities of labelled synapses, the vesicle numerical densities, or the synaptic strength. These results imply that sensory information about food is transmitted more effectively to the GCN in hungry snails than in sated snails.

418.10  BEHAVIOURAL ANALYSIS OF INTERACTIONS BETWEEN TWO ANTAGONISTIC REFLEXES IN C. ELEGANS. C. B. Ramalho. Department of Psychology, University of British Columbia, Vancouver, BC V6T 1Z4

In response to a mechanical tap the nematode Caenorhabditis elegans shows a withdrawal reflex, swimming backwards for some distance. This reflex is capable of habituation and sensitization, as well as both short and long term memory. In addition, we have discovered two antagonistic reflexes in C. elegans. Antagonistic reflexes that use the same final common path cannot be activated simultaneously, therefore, if we fed one group of snails ad lib, while a second group was starved for 5 days. Snays converging from the GCN were identified within the brain, after injecting the GCN with hexamminecobalt. The principal result was that the ratio of flat synapses to flat synapses was significantly greater in the starved animals than in the sated animals. There were no significant differences between the groups in regard to the numerical densities of labelled synapses, the vesicle numerical densities, or the synaptic strength. These results imply that sensory information about food is transmitted more effectively to the GCN in hungry snails than in sated snails.

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In order to investigate the mechanisms underlying a behavior, it is necessary to determine the neuronal elements involved in the behavior. In this research we have focused on the neural circuit underlying the withdrawal reflex made by the nematode worm C. elegans in response to a mechanical tap. Using mutant analysis and laser ablation techniques we have determined that the touch withdrawal circuit described by Chalfie and colleagues also mediates the tap withdrawal reflex. Our behavioral experiments showed that the tail-touch withdrawal reflex is inhibited by the tap withdrawal reflex. Injections into how these antagonist responses can be reconciled within the touch circuit followed three lines. First, mutants specific for identified neurons within the circuit have been identified. For example, dpy-11 is a mutant in which the interneuron PVC is afferent to PVC. Second, laser ablation of individual neurons within the circuit confirmed and extended the genetic studies. Finally, many of the worms neurons are postembryonically. That the worm shows inhibition prior to the formation of some synapses argues against the possibility of those synapses mediating the response.


Caenorhabditis elegans is a simple soil dwelling nematode that exhibits sexual dimorphism. The male C. elegans is differentiated from the hermaphrodite by the additional structures in the tail. In this study, we compared the behavioural responses of the male to that of the hermaphrodite. It was hypothesized that the anatomical differences in mechanosensory structures might result in behavioural differences in the responses to vibratory stimulation (a tap to the side of the holding dish). The adult male C. elegans shows a higher incidence of spontaneous reversals than the hermaphrodite. In response to differing intensities of tap there were no apparent differences between the responses of males and hermaphrodites. Further, the male was capable of simple non-associative learning; it demonstrated habituation, disinhibition and recovery from habituation in much the same manner as the hermaphrodite. Tail-touch induced inhibition of the reversal response appeared to be similar in males and hermaphrodites.

Thus, there appeared to be no behavioural differences between the male and hermaphrodite in the tap withdrawal reflex despite the presence of additional neuronal structures in the male.


Pairing and predictive relationships between stimuli are critical variables in associative learning for both vertebrate and invertebrate species including the leech, Hirudo medicinalis. We previously reported that the Krouzus cell (R) may be implicated in the phenomena (1985, Soc. Neurosci. Abs., 14, 838). That is, the R cell, driven by the US, continues to fire to throughout training for leeches experiencing paired CS-US presentations. In contrast, the R cell decrements to repeated US alone presentations as well as to paired CS-US presentations with added unpaired US alone trials. Learning only occurs following paired training.


In Caenorhabditis (C.) elegans, there are changes with age in habituation and recovery from habituation. Worms were tested at 4 days (peak of egg-laying), 7 days (after completion of egg-laying), and 12 days (shortly before death) of age. While the frequency of reversal withdrawal responses to single taps to the holding dish (vibrational stimulus) did not change with age, the magnitude of the reversals diminished. In post-reproductive development, worms at all ages habituated to trains of taps delivered at a 10s interstimulus interval. However, worms tested at 7 and 12 days continued to make small responses throughout stimulation while 4 day olds often stopped responding entirely. All ages showed habituation in response to a mild electric shock. However, spontaneous recovery from habituation (measured 30s, 1min, 2min and 10min after the last habituating stimulus) was seen only in the worms tested at 4 days and 7 days. The deficit in recovery at 30min in worms 12 days of age cannot be accounted for by motor fatigue as worms of the same age habituated after habituation training. Describing the changes in non-associative learning with age in normal wild-type worms will allow an analysis of the effects of treatments which alter the aging process in C. elegans.


The neural bases of several behaviors have been characterized in the adult medicinal leech. As a preliminary to studying the development of these behavioral circuits, we have been examining the normal course of behavioral development in the embryos between developmental days 10 and 31. We have identified several sensory structures in the embryos and characterized different, both spontaneous and evoked, using microscopic observations and microcinematography with frame-by-frame analysis of behavior. We have found that the behavioral development along a consistent course. For instance, spontaneous lateral movements of the head appear earlier than shortening can be evoked, and both appear to be required for the establishment of walking. There is a neuroanatomical gradient of development for some behaviors, particularly the ones, such as lateral bending, which develop first. Complex behaviors develop in stages. For instance, in the development of crawling, the embryo masters the coordinated elongation and contraction phases of the step cycle before it can use the front and rear suckers toassociate and complete the cycle. Further, as is the case in other poikilotherms, the overall rate of development depends on temperature. We are investigating whether the sequence of behavioral development is also temperature-dependent. This work was supported by USPHS research grant MH43996 to WKB.


Shortening, a defensive reflex of the leech, exhibits disinhibition and sensitization in response to a noxious stimulus. Chain of coupled interneurons (S cells) that forms the Fast Conducting System of the leech has an uncertain role in this reflex. In this study an intracellular pronase injection was made in two single S cell in segmental ganglia 1 to establish the role of the S cell in sensitization and in disinhibition of the shortening reflex. Control leeches underwent surgery without S-cell pronase injections. The effect of the lesion was assessed in sensitization of S-cell killing using the protocols described previously. The role of the S cell in sensitization and in disinhibition of the shortening reflex is added evidence that these types of learning may operate by different mechanisms.

Supported by USPHS Grants NRB 10097 (BM), NIH ROI-NS26007 (RM) and the Whitehall Foundation and ROI-MH 44789 (CS).
418.17  GIANT FIBER ACTIVATION OF AN INTRINSIC MUSCLE IN THE MESOTRHOSTAL LEG OF DROSOPHILA. J.B. Trimarhe and A. Schneider-Champion. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

Many of the muscles and neurons recruited during the escape response in Drosophila are critical for the execution of escape. In particular, the tergotrochanteral muscle (TTM) which adducts the femur provides the force which propels the fly from its substrate during the initial jumping phase of this behavior. (Mulloney, 1969, J. Verg. Physiol., 64: 243-253). The present study focuses on the synergistic role in jumping of the tibial lateral fiber (TL), an intrinsic leg muscle.

During activation of the escape response by stimulation of the giant fiber pathway (Tanouye & Wyman, 1980, J. Neurophysiol, 44: 405-421), muscle results from the TL with characteristic latencies of 1.3 ± 0.01 msec. They occur 0.48 ± 0.04 m sec after muscle potentials appear in the TTM and the dorso longitudinal indirect flight muscles (DLMs), respectively. The threshold voltage of brain stimulation required to elicit a response in the TL is the same as that required for the TTM and DLMS. As stimulus voltage increases, the responses of the TTM and DLMS undergo a distinctive shift from short to long latency (Levines & Tracy, 1973, J. Comp. Physiol. 87: 213-225). We observed that the response of the TL maintains its temporal delay with respect to the responses of the TTM and DLMS during this shift.

HRP backfills of the TL motor neuron indicate that it has a dendritic arborization in the leg neuromere and a medially directed neurite. We are extending these studies to characterise the neurite morphology underlying activation of the TL by the giant fiber pathway. (NSF BNS-90-09863, NIH 5-T32-GM07469)

418.19  SUPPRESSION OF ACTIVITY-DEPENDENT SYNAPTIC PLASTICITY IN NEURAL TERMINAL ARBORIZATION BY MEMORY MUTANTS IN DROSOPHILA: L. Boynton and C.-Y. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Previous studies on hyperactive and dnc mutants suggest that the cAMP cascade is a crucial biochemical pathway in mediating activity-dependent enhancement of motor neural terminal arborization in Drosophila larvae (Budnik et al., 1990, J. Neurosci. 10: 3754; Zhong et al., 1990, Neuron, 5: 437). We investigated the effect of the rutabaga (rut) mutation, which eliminates Ca/CaM activation of adenylyl cyclase, on hyperexcitability mutation and temperature-induced enhancement in larval motor terminal arborization.

In larvae reared at 25°C, the numbers of motor terminal branches and varicocities were increased in hyperexcitable Shaker (Sh) mutants with a reduced or eliminated transient K current (mean±SD of branches/varicosities in fiber 12 and 13, abd. segment 3, from 8-12 larvae: 418.21  THE RHYTHMIC MOTOR PATHWAY CONTROLLING OVIDUCT CONTRACTIONS IN LOCUSTA MIGRATORIA: DESCENDING NEURAL CONTROL AND TRACING OF EFFERENT TARGETS. G. Falcone* and A. B. Lange, Dept. of Zoology, University of Toronto, Etendale College, 3353 Mississauga Road, Mississauga, Ontario, Canada. L5L 1C8.

The oviduct and associated muscles of the reproductive system of Locusta migratoria is regulated by the Vth abdominal ganglion which project along the oviductal nerves (OVNs). Interruption of Locusta dorsal-root transection results in rhythmic activity recorded extracellularly from the OVN.

The rhythmic neural activity consists of up to five discernable different patterns and no apparent fraction. All units fired with temporally interrelated patterns. The motor pattern persists in isolated Vth abdominal ganglia of disturbed epithelial preparation of a motor pattern generator. In ovo-laying females, where neural activity at the OVN is normally very low, the rhythmic motor pattern was initiated by transection of the Vth abdominal connectives. Units within the motor pattern were traced to their respective muscle targets. One unit recorded from a branch of the OVN innervating the Vth lateral and common oviduct. Other were recorded from branches of the OVN innervating skeletal muscles in the Vth abdominal segment lying in close association with the ovipositor muscles. The motor pattern therefore coordinates the activities of both a visceral muscle and skeletal muscle groups. These results suggest that contractions of the lower lateral and common oviduct as well as associated skeletal muscles are controlled, at least in part, by a pattern generator located in the Vth abdominal ganglion. These circuits appear to be under descending inhibitory control with the inhibition being removed during the disruption of ovo-laying.

418.18  EFFECTS OF CAMP ON GROWTH CONE MOTILITY IN CULTURED CNS NEURONS: ANALYSIS OF DROSOPHILA MEMORY MUTANTS, dunce AND rutabaga. Sherry S. Chang Wu. Department of Biology, University of Iowa, Iowa City, Iowa 52242.

Learning and memory capacity of animals depends on cellular mechanisms underlying functional plasticity of nervous systems. This plasticity may involve morphological modifications of nerve terminal arborization and synaptic contact between neurons. The growth cone may be a specific link between mutations. Since dnc and rut affect CAMP-specific phosphodiesterase and Ca2+-calmodulin activation of adenylyl cyclase, respectively, we examined the effects of 10-50 μM forskolin or dibutyryl CAMP in the perfusion medium. The treatment resulted in retraction and thickening of lamellipodia which led to lower motility and triggered the transitory lamellipodia into club-shaped neurite endings. The fact that either elevated or lowered CAMP in dnc or rut exhibited similar effects on growth conest suggests that an optimal level of CAMP is required to maintain growth cone motility, which may be important to neural plasticity underlying learning behavior.

418.20  LATHOE, A NEW SINGLE GENE MUTANT AFFECTING LEARNING AND MEMORY IN DROSOPHILA MELANOGASTER. S. Boynton and T. Tully. Department of Biology, Brandeis University, Waltham, MA 02254.

We have begun a screen for new mutations affecting classical conditioning or olfactory avoidance responses in Drosophila. Use of P-elements as the mutagenic agent will ultimately explicate molecular cloning of the relevant genes. One strain isolated from this mutagenesis, lathoeP1, shows reduced learning and memory. Behavioral analyses indicate that the behavioral deficit of lathoeP1 flies is not caused by an inability to smell the odor (conditioned stimulus) used during training or by an inability to sense and escape from electric shock (unconditioned stimulus). Genetic analyses of lathoeP1 indicate that the behavioral deficit in lathoe flies is attributable to a single gene. Flies heterozygous for the lathoeP1 insertion mutation and for chromosomal deficiencies of the second chromosome show abnormal memory of olfactory avoidance conditioning. Two more alleles of lathoe were lethal, and the lethality mapped to a single, lethal complementation group in the vestigial region. More severe alleles of lathoe show pleiotropic effects on several behaviors reflecting the fact that the normal lathoe gene product is necessary for survival. In particular, locomotor activity was reduced in lathoeP1 flies hematogous for lathoeP1 or lathoeP1 flies heterozygous with a lethal allele. The isolation of several hypomorphic excision alleles, however, allows a specific link between lathoe and memory/learning to be established. Supported by grants from NIH (GM 32005), the McKnight Foundation, the John Merck Fund, and HHMI (MH0994).


We examined the effects of three different stimuli (sucrose solution to the antenna, to proboscis, or to both) on the response to an odor (carnation) in a sensitization paradigm. Responses to the control stimulus (carnation), the sensitizing stimulus (sucrose solution), and test stimuli (5, 1, 2, 5 min later) were monitored by extracellular recording from a muscle involved in proboscis movement. The number of muscle spikes per stimulus served to judge the response intensity. Also, the percentage of successfully sensitized animals was compared among the different experimental groups. During the control group, we could discriminate animals which showed a response (sensitized animals) and nonresponsive animals (not sensitized). In both groups a sensitizing effect was apparent with either treatment at the test trial at 5 min, but among the different experimental groups we found no differences for spontaneous animals. The results reported below refer to non-sensitized animals.

At the first test trial the percentage of successfully sensitized animals was significantly greater when sensitized by antennal stimulation. In contrast, the response-intensity (i.e. muscle spikes per trial) to the sucrose solution stimulus and during the first test trial was lowest. The experimental groups receiving stimulation to proboscis or to both proboscis and antenna did not show profound differences. The combined results reveal that intensity and probability of the sensitizing effect are not linked in a trivial way. As a functional interpretation we suggest that antennal sensitization facilitates the sensory components of the reflex, while the procedures involving stimulation of the proboscis facilitate components of the motor program.
418.23 DIFERENTIAL TONIC INHIBITION OF CRAYFISH LATERAL GIANT ESCAPE TO STIMULI IN DIFFERENT SENSORY FIELDS. F.B. Krasne, E.T. Vu, and S.C. Lee. Psychology Department, University of Wisconsin, Madison, Wisconsin. Tonic inhibition of crayfish lateral giant escape reflex is mediated by a "tonic inhibition" pathway that descends into the abdomen from more rostral ganglia. Recent physiological evidence (Vu and Krasne, these abstracts) indicates that tonic inhibition is due to remote postsynaptic inhibition of the lateral giant command neurons. Remote postsynaptic inhibition theoretically has the capability to inhibit a selected region of a dendritic tree. A preliminary test of whether the tonic inhibition pathway can suppress responses to selected stimuli fields, we simultaneously monitored restraint-induced inhibition in two different abdominal segments to see if differential onset or offsets could be observed. We found that inhibition tends to turn on at about but not exactly the same time in different segments. Thus, the different segments are subject to independent control. Experiments to search for differential inhibition of different fields within a hemisegment are planned. Supported by USPHS grant NS08108 (FX) and a Product. NSF Fellowship (EV).

418.24 OCTOPAMINE INCREASES EFFICIENCY OF COMMISSURAL TRANSMISSION BETWEEN CRAYFISH LATERAL GIANTS. S.C. Lee and F.B. Krasne. Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024. Octopamine is known to increase transmission between primary afferents and first order interneurons of the crayfish lateral giant escape reflex circuitry and has been conjectured to mediate behavioral sensitization of the lateral giant escape reflex. Evidence that it also affects the lateral giant reflex has not been provided by the finding that octopamine (8×10^{-5} - 3×10^{-7} M) decreases the time needed for one lateral giant to recruit its contralateral homologue by up to 20%. This effect is antagonized by the octopamine antagonist, phentolamine (10^{-5} M). The presence of functional octopamine receptors on LGs may facilitate analysis of octopamine's mechanism of action in this system. Supported by USPHS grant NS08108 (FX).

419.1 THE ONTOGENY OF BEHAVIORAL ASYMMETRY IN THE RAT: INFLUENCE OF SEX & PREGNATAL TESTOSTERONE. K.J. Schultz and J. Quinter. Psychology Department, University of Wisconsin, Madison, Wisconsin. Behavioral asymmetry exists in rats and tends to be more strongly exhibited by females than males. Tail posture asymmetries assessed at Day 1 (birth=Day 0) have been found to be predictive of adult turning preference. This study assessed the development of behavioral asymmetries from Day 1 to Day 21 in male (N=44) and female (N=52) Sprague-Dawley rats with varying levels of prenatal testosterone. Asymmetries assessed at Day 1 (birth=Day 0) have been found to be predictive of adult turning preference. This study assessed the development of behavioral asymmetries from Day 1 to Day 21 in male (N=44) and female (N=52) Sprague-Dawley rats with varying levels of prenatal testosterone. Testosterone levels were inferred from anogenital (AG) distance measurements taken on Day 0. The development of behavioral laterality was evaluated using measures of tail posture and negative geotaxis turning direction, while adult patterns of behavioral laterality were assessed on a circling, a swimming and a head turn task. Laterality measures taken on Day 1 revealed no turning preferences and did not reliably predict adult laterality. A rightward turning preference did emerge over the next two weeks, particularly for negative geotaxis, and over this period tail posture varied with sex and AG level as well as age. Measures taken on Days 3 to 15 were significant predictors of adult turning preference and adult behavior also varied as a function of sex and AG distance. Neural mechanisms underlying these changes are being explored.

419.2 RAPID NEUROPHYSIOLOGICAL ACTIONS OF CORTICOSTEROINE RELATED TO STRESS-INDUCED INHIBITION OF SEXUAL BEHAVIOR IN AN AMPHIBIAN. J. D. Rose, F. L. Moore and M. Orchinik. Dept. of Psychology, University of Wyoming, Laramie, WY 82071 and Dept. of Zoology, Oregon State University, Corvallis, OR 97331. Acute stress inhibits courtship clasping by male rough-skinned newts due to a rapid effect of corticosterone (CORT). A binding site in neuronal membranes from newt brain, with properties correlated with the CORT inhibition of clasping has recently been characterized (Orchinik, Murray and Moore, Science, in press.) In the present study, neural actions of CORT were examined in male newts by microluteospinal neurons that were backfired (androgenically-activated) by spinal cord stimulation. Systemic administration of a behaviorally-effective dose of CORT produced an increase in antidromic spike amplitude within 5 min that was followed immediately by a progressive decline in excitability, but more rapidly than systemically-administered pads. We have examined plasma steroid levels and D.L. Hess*. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112, and Oregon Reg. Primate Ctr, Beaverton, OR 97006. Four species of Bornean frogs lack a suite of behavioral and morphological secondary sex characteristics which occur in breeding north temperate and tropical frogs (enlarged forelimbs, advertisement calls, vocal sacs, nuptial pads). We have examined plasma steroid levels in reproductively active males of one species of voiceless frogs (B. blanchardi) to test the hypothesis that decreased circulating androgens could be at least part of the explanation for the unusual loss of these characteristics. Frogs were captured in Sabah, Malaysia, in 1989 and 1990 and immediately bled. Plasma androgens were lower in B. blanchardi than reported for other species in the literature where comparable blood sampling techniques were used. We suggest that lowered androgen levels may be at least part of the explanation for the loss of behavioral and morphological secondary sex characteristics in these frogs. [NSF grant #BSR-8822630 (SBE) and NIH grant #HD18185 (DH)]

419.3 STEROID HORMONE MEDIATION OF THE EFFECTS OF ENERGETICS ON MALE FROG REPRODUCTIVE BEHAVIOR. C.A. Marler, M. Ryan and W. Wilczynski. Deps. of Zoology and Psychology, University of Texas, Austin, TX 78712. Energetic factors might influence reproductive success in male newt frogs (Physalaemus pustulosus) by limiting the best predictor of male mating success: sexual quiescence. We manipulated food intake and found that males with lower food intake called for fewer hours, even though their lipid reserves were not greatly decreased. Average lipid stores in males provided energy for calling approximately six hours a night for six nights. Hormonal mechanisms potentially mediating this effect of energetics on behavior were also examined. One potential mechanism for decreasing calling is through lower testosterone (T) levels, possibly through increases in corticosterone (C) levels. Males with lower food intake had lower T-levels. Male reproductive behavior was associated with T-levels: calling males had higher T-levels than noncalling males. Two lines of evidence suggest that B might influence T levels: (1) B-levels were negatively correlated with T-levels, and (2) B-implants caused a decrease in T-levels. B may inhibit reproductive behavior directly. B-implants decreased calling behavior, but there was no difference in B-levels between fed and unfed males. However, levels were high in all groups, possibly masking a difference. These data suggest that plasma steroid levels may be mediating effects of energetic factors on reproductive behavior. (Supported by Smithsonian Tropical Research Institute and NIH grant T32 MH1837-01)

Male Xenoecus laevis frogs produce a song consisting of alternating fast and slow trills while adult females do not. We examined the ability of testicular hormones to induce singing in females. Adult male and female frogs were implanted with Silastic tubes which contained either testosterone propionate (TP) or nothing, and then tested after 13-17 months with sexually unreceptive females. While TP restored singing in castrated males, it did not induce singing in overtampering females. Since previous studies have shown that long-term TP treatment fully masculinizes laryngeal muscle contractile properties, these female's larynges were capable of song. Male and female frogs from different populations from metamorphic to early adulthood (20-50.0 g B.W.) were gonadectomized and implanted with testes, grown to sexual maturity (as evidenced by growth of nuptial pads), and tested for singing. All testes-implanted females sang. The number of songs and total duration of singing were not significantly different from identically-treated or intact adult males. Females implanted early in post metamorphic development produced fully masculinized trills (53-70 Hz), but females implanted after 15 g B.W. produced trills which were slower (45-50 Hz). This study provides the first evidence that testicular hormone is necessary in female X. laevis. Supported by NS23684 and NS08304.

419.7 DISASSOCIATION OF JUVENILE PLAY FIGHTING AND ADULT DOMINANCE IN MALE RATS TREATED NEONATALLY WITH TESTOSTERONE PROPIONATE. S. M. Pellis and V. C. Pellis. Dept. Psychology, Univ. Lethbridge, Lethbridge, AB. T1K 3M4

It is known that juvenile female rats play less than juvenile males and that neonatal treatment with testosterone propionate (TP) elevates the frequency of play of females to that of males. Such treatment to males increases their play to levels above that shown by controls. Studies have indicated that neonatal exposure to androgens have a masculinizing effect on brain and behaviour. In the present study, neonatal male rats were injected with TP (two injections of 0.05 ml each, one/day, of 250 μg/ml sc. in the nape) and a control group received an equivalent volume of the oil vehicle. At weaning, each injected male was paired with a male littermate, and they were housed together until 100 days old. At 30-40 days and at 80-90 days, they were isolated from one another for 24 h and then re-paired in a test enclosure, and their play behaviour was videotaped. This was repeated twice at each age. At the 30-40 day age class, TP treated rats played 2-3 times more frequently than the oil injected controls. This is comparable to other studies. When housed together, male pairs form a dominant-subordinate relationship which becomes more clearly asymmetrical in the weeks following sexual maturity (i.e., 50-60 days). Behavioural and physical measurements at 80-90 days clearly identified the dominant pairmate in each pair. TP treated rats were no more likely to become the dominant pairmate than were their untreated pairmates. Therefore, while neonatal exposure to androgens masculinizes play behaviour, it does not appear to influence other masculine behaviours related to aggression and dominance.


The nucleus intercollicularis in quail is implicated in the control of a number of vocalizations. It is also a target structure for testicular testosterone (T). TP elevates the frequency of play of females to that of males. Such treatment to males increases their play to levels above that shown by controls. Studies have indicated that neonatal exposure to androgens have a masculinizing effect on brain and behaviour. In the present study, neonatal male rats were injected with TP (two injections of 0.05 ml each, one/day, of 250 μg/ml sc. in the nape) and a control group received an equivalent volume of the oil vehicle. At weaning, each injected male was paired with a male littermate, and they were housed together until 100 days old. At 30-40 days and at 80-90 days, they were isolated from one another for 24 h and then re-paired in a test enclosure, and their play behaviour was videotaped. This was repeated twice at each age. At the 30-40 day age class, TP treated rats played 2-3 times more frequently than the oil injected controls. This is comparable to other studies. When housed together, male pairs form a dominant-subordinate relationship which becomes more clearly asymmetrical in the weeks following sexual maturity (i.e., 50-60 days). Behavioural and physical measurements at 80-90 days clearly identified the dominant pairmate in each pair. TP treated rats were no more likely to become the dominant pairmate than were their untreated pairmates. Therefore, while neonatal exposure to androgens masculinizes play behaviour, it does not appear to influence other masculine behaviours related to aggression and dominance.

419.10 LOCALIZATION OF MOTORNEURONS INNERRATING THE MAJOR MUSCLES OF THE CLOACA IN JAPANESE QUAIL. C. M. Sciret and E. A. Alakia. Department of Psychology, Cornell University, Ithaca, N.Y. 14853.

Four major striated muscles are associated with the dorsal cloaca in birds: the sphincter cloacae (mSC), the major cloacal muscle (mMC), the minor cloacal muscle (mMC), and the constrictor cloacae (mCC). By the largest of these is the sexually dimorphic, hormone-sensitive mMC. Motorneurons responsible for the innervation of mMC lie in the lateral motor column of somatic segments 8, 9, and 9 or 8, 9, and 10 (Sciret and Alakia, 1987). The variability in rostral-caudal location reflects individual differences in which segments of the cord contribute to the pudendal nerve. We report that the motorneurons supplying mMC and mMC also lie in the lateral motor column in the same segments as those innervating mMC. A single small injection of cholera-toxin conjugated to horseradish peroxidase (CT-HRP) was made into the dorsal musculature of the cloaca (mMC-0.5 μl, n=7; mMC-0.3 μl, n=8; mMC-0.25 μl, n=2; mMC-0.25 μl, n=3). Animals were sacrificed 48-48 hours later. Raguest of the muscle injected, labelled somata with multiple granules in the lateral motor column of somatic segments 7, 8, 9 or 9, 8, and 10. Bilateral experiments were observed into mMC and mMC, while in mMC and mMC labelled somata were present only bilaterally to the injection site. No differences existed in the proportion of labelled somata present between the 2 groups. Thus the motorneurons which innervate mMC, mMC, and mMC are distributed throughout the larger pool of motorneurons innervating mMC. Work in progress addresses the following questions: 1) Does sex differentiate in muscle fiber type in cloacal musculature? 2) Are clonal motor neurons, like cloacal muscle, sensitive to sex steroids? [Supported by NSF # BSR-86-09441].

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Erection is controlled by the pelvic nerve (PNK) while hypogastric nerve (HGN) has an inhibitory function. Combined electrical stimulation of both nerves results in penile erections, whereas stimulation of each nerve by itself results in penile reflexes. PNK stimulation of the dorsal nerve of the penis (DNP) results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduces the penile responses. This inhibitory function of the HGN is more pronounced when compared to the PNK, and it is not affected by the dose of electrical energy. The combined electrical stimulation of both nerves results in erections that are augmented proportionally to the dose of electrical energy, whereas the contralateral HGN has an inhibitory function that reduce
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MOTONEURONS OF THE GUINEA PIG RETRATOR PENIS ARE SEXUALLY DIMORPHIC IN SIZE BUT NOT NUMBER. Louise M. Freeman & S. Marc Breedlove. Psychology Department, Univ. Calif., Berkeley CA 94720.

The retrator penis (RP) attaches between the penis and the pubis in male guinea pigs, and this much smaller counterpart, the rector or citius (RC), is present in females. We previously reported that the associated motoneurons occupy the central region of the ventral horn from the L5 to S1 regions of both sexes (Freeman & Breedlove, Soc. Neurosci. Abstr. 16:4, 1990). To determine whether these motoneurons were sexually dimorphic in number or size, we counted and measured cells labeled by large muscle injections of horseradish peroxidase (HRP) into the muscle. Under ketamine anesthesia, six adults of each sex were injected bilaterally with HRP. All HRP-labeled motoneurons were counted, and raw counts were corrected for split nucleic error by the method of Konigsmark. Nuclear and soma area were measured from camera lucida drawings of 12 motoneurons/animal.

SOMA AREA (μm²) 

Males 1250±180
Females 568±127
ANOVA shows a sex difference in MN size (p < .002) but not number (p = .413). The presence of a sex difference in size but not number is in agreement with previous reports (Freeman & Breedlove, Psych. Soc. Neurosci. Abstr. 16:4, 1990).

Supported by NIH NS28421.

419.19


The spinal nucleus of the bulbocavernosus (SNB), spanning L5 and L6 of the spinal cord, innervates perineal muscles involved in penile reflexes. Castration decreases the reflexes (Hart, 1967) and changes the morphology of motor neurons (Breedlove & Arnold, 1981; Kurz et al., 1986). To begin an investigation of the site of androgen action for these effects, we examined the concentration of various androgens required to maintain penile reflexes in castrated rats. Osmotic mini-pumps attached to intrathecal catheters administered testosterone (T) to the lumbar spinal cord in castrated adult males. T treatments were 4.8, 9.6, and 14.4 μg/day for two weeks. The 4.8μg/day dosage was not sufficient to maintain the reflexes, whereas the animals receiving 14.4μg/day displayed reflexes comparable to pre-operation levels. Animals receiving 9.6μg/day of T displayed an intermediate response level. These results indicate that the T concentration necessary for maintaining these behaviors may be higher than previously suggested.

Currently underway is a study of the efficacy of intrathecal versus systemic T administration. In addition to gathering behavioral data, we will examine whether the various T treatments affect the morphology of SNB cells.

Supported by NIH NS28421.

419.21

PASSIVE ELECTRICAL PROPERTIES OF BULBOSPINOSOUS MOTONEURONS IN GONADALLY INACT AND CASTRATED ADULT MALE RATS. W.F. Collins, III. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

Bulboaspinous motoneurons (BS MNs) are smaller in castrates, as compared to gonadally intact, adult male rats (Kurz et al., Science 232:595, 1980). The present study was undertaken to evaluate possible changes in the passive electrical properties of BS MNs following castration. Ovariectomized and castrated Sprague-Dawley rats (350-400 gm) were anesthetized and in vivo intracellular recordings were obtained from BS MNs (1.6-4.4µA) using conventional techniques. All lumbar and sacral dorsal roots were cut bilaterally. Passive membrane properties were assessed by injecting 1 nA of hyperpolarizing current (30-50 ms) and averaging (n=32-64) the resulting change in membrane potential. Following correction for anomalous rectification, input resistance (Rm) was measured. Estimates of membrane (Tm) and equalizing (Tt) time constants were made from the voltage transient following cesation of the current and used to calculate the electronic length (L). The data were analyzed using a two-level nested ANOVA, and the results are summarized below. Although mean Rm was larger in castrates, no significant difference was obtained for any of the parameters. These results indicate that the passive electrical properties of BS MNs are relatively unaffected by castration. Supported by NIH NS4206 (WFC) and NS16996 & NS14899 (L.M. Mendell).

<table>
<thead>
<tr>
<th>Group</th>
<th># MNs</th>
<th>Tm (ms)</th>
<th>Tt (ms)</th>
<th>L (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>13</td>
<td>2.46 (0.42)</td>
<td>3.51 (0.43)</td>
<td>0.48 (0.08)</td>
</tr>
<tr>
<td>Castrate</td>
<td>19</td>
<td>3.29 (0.46)</td>
<td>3.71 (0.48)</td>
<td>0.59 (0.08)</td>
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</tbody>
</table>

419.22

FEMALE HAMSTER VAGINAL SECRETION STIMULATES C-FOS EXPRESSION IN THE VOMERONASAL AND OLFACTORY MATING BEHAVIOR PATHWAYS IN THE MALE GOLDEN HAMSTER. E.J. Calabrese & J.W. Severson. Dept. of Biological Sciences, Rutgers University, Newark, N.J. 07102.

Olfactory and vomeronasal chemosensory input is essential for arousal and execution of mating behavior in male golden hamsters. Preovulatory anovulatory investigatory behavior exerts the male to Female Hamster Vaginal Secretion (FHVS). Male hamsters exposed to FHVS for 1 hour elicit exploratory and investigative behavior. Lesioned sites included the raphe, parametrical (PVN), caudal and rostral aspects of the mesencephalic reticular formation (MRF), and the medial preoptic area. Moreover, the olfactory bulb is essential for the expression of these mating behaviors. Lesioned animals were able to respond to male hamsters exposed to FHVS (L. M. Breedlove & E. P. Monaghan, Soc. Neurosci. Abstr. 16:4, 1990). These findings provide further evidence for the importance of the olfactory bulb and olfactory system in the expression of these behaviors.

In the current experiment we determined which brain areas were stimulated by FHVS using c-fos immunocytochemistry as a marker of cell stimulation. Male castrated and intact hamsters were exposed to FHVS for 1 hour following castration. Animals were sacrificed 3 days after castration and used to calculate the electronic length (L). The data were analyzed using a two-level nested ANOVA, and the results are summarized below. Although mean Rm was larger in castrates, no significant difference was obtained for any of the parameters. These results indicate that the passive electrical properties of BS MNs are relatively unaffected by castration. Supported by NIH NS4206 (WFC) and NS16996 & NS14899 (L.M. Mendell).

419.18


Motoneurons in the spinal nucleus of the bulbocavernousus (SNB) innervate striated perineal muscles which mediate reflexive cup-like erections of the penis. Midthoracic spinal transections potentiate these reflexes, indicating that the different regions of the spinal cord may have different roles in mediating penile reflexes. Lesioned sites included the raphe, paraventricular (PVN), and lateral vestibular (LNV) nuclei of adult male rats. Ablation of the medial raphe nucleus (n=8) increased the display of penile cups and flips (p<.02). PVN lesions (n=6) increased the latency to the first erection (p<.05) but did not otherwise affect penile reflexes. In animals with complete lesions of the LNV (n=4), penile reflexes were eliminated, however these lesions had a generally debilitating effect on the animals. These results suggest that the raphe, LNV, and PVN provide spinal projections which influence the expression of penile reflexes.

Supported by NIH NS28421.
419.23
UNILATERAL LESIONS OF THE SEXUALLY DIMORPHIC AREA (SDA) OF THE GERRILL HYPOTHALAMUS DISRUPT MALE SEXUAL BEHAVIOR WHEN COMBINED LESIONS OF THE MEDIAL ANGIOLOY-ANGUICANTICULAR AREA (MA-AH) OR MEDIAL NUCLEUS OF THE LATERAL HYPOTHALAMUS (MEN) IN MALE RATS.

420.1
FUNCTIONAL CHANGES INDUCED BY HABENULA CELL TRANSPLANTS FOLLOWING LESION OF THE FASCICULUS RETROFLEXUS.

420.2

420.3
EFFECTS OF OPIATE-AMPHETAMINE CROSS-SENSITIZATION IN CONDITIONED REINFORCEMENT ARE MEDITATED BY M- BUT NOT DELTA-SELECTIVE OPIATE RECEPTORS IN THE NUCLEUS ACCUMBENS. S.T. Cunningham and A.E. Kelley, Dept. of Psychology, Northeastern University, Boston, MA 02115.

420.4
REGULATION OF VENTRAL PALLIDAL-STIMULATED LOCOMOTION BY THE NUCLEUS ACCUMBENS DEPENDS ON ENKEPHALIN AFTER DOPAMINE DEPLETION. L. Churchhill and P.W. Kalivas. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

NEUROPEPTIDES AND BEHAVIOR I
4.20.5 DIFFERENTIAL EFFECTS OF A CCK4 AND CCK8 ANTAGONIST AGAINST MORPHINE CONDITIONED PLACE PREFERENCE (CPP). G.A. Higgins and P.M. Selmers. Departments of Pharmacology, Medicine and Psychiatry, University of Toronto and Clinical Research and Treatment Institute, Addiction Research Foundation, Toronto, Ontario, Canada M5S 2S1.

Experimental evidence consistently supports involvement of the dopamine (DA) mesolimbic pathway in behaviours underling drug-induced reward (e.g. morphine CPP). CCK is also closely associated with this system and CCK antagonism may modulate a number of DA dependent behaviours. We have previously reported (Higgins et al., Eur J Pharmacol, 1991 [in press]) that pre-treatment with CCK8 antagonist, devazepide, blocked the acquisition of a morphine CPP. In the present report we describe the effects of L366-260 (L), a selective CCK antagonist, on this behaviour. (L) was administered (0.001-10 μg/kg i.c.v.) 30 min prior to morphine (1.5 mg/kg i.c.v.). The procedure was as described previously (Higgins et al., Eur J Pharmacol, 1991 [in press]). At no dose did (L) block morphine CPP, instead a mild potentiation was recorded. To study this further we examined the interaction between (L) (10 μg/kg) and a subthreshold dose of morphine (0.3 mg/kg). At these doses neither drug elicited CPP alone, however, when co-administered, a CPP was recorded (p < 0.01). In addition, a higher dose of (L) (1 mg/kg) also induced a significant CPP when administered alone (+120 ± 45 sec, p < 0.05).

It is concluded that antagonism of CCK8 receptors may attenuate a behaviour indicative of opioid reward, while antagonism of CCK subtype potentiates this response. These effects might be related to the differential roles of these receptors on DA release within the nucleus accumbens (Marshall et al., J Neurochem 56: 917-922, 1991).

4.20.6 MEDIAL PREOPTIC AREA CHOLECYSTOKININ INFUSIONS BLOCK Beta-ENDORPHIN INDUCED INHIBITION OF MATERNAL BEHAVIOR IN LACTATING RATS. P.E. Micewch, P.H. Hinton, and R.S. Bridge. Comparative Medicine, Tufts University School of Veterinary Medicine, North Grafton, MA 01536.

Recent work has demonstrated that intraventricular infusions of cholecystokinin octapeptide (CCK-8) prevents the disruptive actions of beta endorphin (βE) on maternal behavior in lactating rats. Maternal care is also blocked when βE is infused into the MPOA. In the present study we investigated the possibility that CCK-8 might antagonize βE's action on maternal behavior at the level of the MPOA. Day 5 6-lactating rats previously implanted with microinjection cannulas in the MPOA were given either βE (200 μg) or βE plus CCK-8 (1 μg) on day 5 with saline and on Day 6 with βE (0.145 nmol) or βE plus CCK-8 (1.45 nmol). Testing for maternal behavior began 30 minutes after each infusion and responses were scored throughout the 1 hr test session against 1 hour of daily intervals until all females were fully maternal. Approximately 50% of the βE plus CCK-8 rats were fully maternal within 60 min., whereas none of the βE alone rats responded during this period. In addition, animals treated with βE plus CCK-8 had significantly shorter latencies than βE animals to retrieve, group, and crouch over their pups. These results demonstrate that the administration of CCK-8 directly to the MPOA blocks the inhibitory actions of βE, and suggest that the MPOA is one site of dual peptide regulatation of maternal behavior. [Supported by NIDA Grant #DA04291 to RSB].

4.20.7 ESTROGEN REGULATION OF PREPROENKEPHALIN-A IN TACHYKININ-CONTAINING CELLS IN THE RAT VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. M.A. Pates, P. Popper, and P.E. Micewch. Dept. of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Regulation of lordosis behavior by the ventromedial nucleus of the hypothalamus (VMN) involves the interaction of estrogen with steroid-sensitive neuropeptide circuits, some of which contain enkephalin and the tachykinin substance P (SP). Estrogen induces ENK expression in the VMN but doesn't appear to affect expression of SP, although populations of estrogen-concentrating cells (ECC) have been shown to express SP-like immunoactivity. Additionally, a previous immunohistochemical study (Pates et al., Endocrinology, 1990; 126, 3258-3267) did not identify a population of neurons in the VMN that contained both ENK-like and SP-like immunoactivity. The commercially obtained antisera used in this study, however, showed extensive cross-reactivity with other opiate and tachykinin peptides. To verify the protein content of the double-labelled cells, the current study was performed using the combined techniques of in situ hybridization and immunohistochemistry. Preproenkephalin-A (PPE-A) mRNA was localized to SP immunoreactive neurons of the VMN using a [α-32P]dCTP-labelled single-stranded cRNA probe complementary to the entire coding sequence of the PPE-A mRNA. The probe was transcribed from a 935 bp PPE-A cDNA insert into a pSP64 vector from Dr. Hiroshi Ishikawa (Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan) and a generous gift from Dr. Hiroshi Ishikawa (Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan). The study also examined the quantitative effects of ovariectomy and estrogen replacement on protein expression in the doubly-labelled population of neurons. Supported by NS12220.

4.20.8 ANTIBODIES RAISED AGAINST αCCK-8 and αCCK-8 INDUCED INHIBITION OF LORDOSIS BEHAVIOR. P.E. Micewch, C.A. Priest, R.K. Hinton, and P. Popper. Dept. of Anatomy & Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA, Los Angeles, CA 90024.

Microinjections of αCCK-8 into the ventromedial nucleus of the hypothalamus (VMH) inhibit the display of lordosis behavior in estrogens primed male and female rats (Babcock et al. Psychopharmacology, 1983:419; Eibach et al. Eur J Pharmacol, 1988:55). In a previous study we found (Higgins et al., Eur J Pharmacol, 1991 [in press]) that at no dose did αCCK-8 induce lordosis behavior in castrated rats and therefore its action appears to be mediated by the endogenous opioid system. We have previously indicated that subthreshold doses of morphine (0.3 mg/kg) significantly antagonized the inhibitory effect of αCCK-8 on lordosis behaviour in castrated females (Higgins et al., Eur J Pharmacol, 1991 [in press]). At no dose did αCCK-8 inhibit lordosis behavior of microinjections of exogenous αCCK-8 peptide into the VMH of male and female rats. We examined the effect of αCCK-8 on lordosis behaviour in castrated rats 48 hours after ovariectomy and the response to subthreshold doses of morphine (0.3 mg/kg) on lordosis behaviour in this model of oestrogen deprivation. Treatment with 5α-αCCK-8 had no effect on lordosis behaviour of ovariectomized females, while 5α-αCCK-8 significantly increased lordosis latency in male rats. To examine whether αCCK-8 contains anti-idiotypic antibodies that interact with the CCK receptor, we sequenced in vitro CCK immune response in vivo with 5α-αCCK-8 and then with 5α-αCCK-8. There was no effect on levels of CCK binding in the VMH, suggesting that 5α-αCCK-8 did not displace 125I-αCCK-8 from its binding sites. In a parallel experiment, 125I-αCCK-8 binding as the VMH was attenuated in a dose dependent manner after injection with a cocktail of 125I-αCCK-8 and 1:50, 1:100, 1:200, 1:400 or 1:800 dilutions of 5α-αCCK-8. At no dose did 5α-αCCK-8 binding increase with greater antibody dilution, indicating that 5α-αCCK-8 bound the 125I-αCCK-8 and prevented interaction of the ligand with its receptor. Together, the results of these experiments refute the hypothesis that 5α-αCCK-8 contains sufficiently high titres of anti-idiotypic antibodies to account for its behavioral effects. Supported by NS12220.
ANXIOLYTIC-LIKE EFFECTS OF THE CCK-B ANTAGONISTS LY 262691, anxiolytic, since the tetrapeptide form (CCK-4) induces panic attacks in humans and CCK antagonists have demonstrated anxiolytic-like effects in animal models of anxiety. In this study, two structurally distinct series of potent, selective antagonists for the brain CCK receptor (CCK-B) were assessed for anticonflict effects in a primate model of anxiety. Lever press responses of squirrel monkeys were maintained under a two-component schedule. During the first component, a response during the 3-min interval elapsed; during the second component, every 30th response during the 3-min interval resulted in the delivery of a mild electric shock (punishment or conflict procedure). At 0.3-10 mg/kg, p.o., the CCK-B antagonists LY 262691 and LY 262684 (pyrazoline class) all increased punished responding, with peak increases reaching approximately 150% of control performance levels at the 1.0 to 3.0 mg/kg doses. These doses had little effect on unpunished responding occurring in the alternate component. Agents with clinical efficacy in anxiety have been shown widely to produce increases in punished responding in this type of model. These results suggest that CCK-B antagonists of diverse structural types may be useful in the treatment of anxiety. The lack of suppression of either component further suggests that such treatment could be accomplished without accompanying sedative activity.

HEART RATE CHANGES FOLLOWING ADMINISTRATION OF PUTATIVE PANOCIDOGIC CHEMICALS IN SQUIRREL MONKEYS. J.E. Barrett, M.C. Linden, H.C. Holloway, M.J. Yui, and J.J. Howbert. Dept. of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and Lilly Research Labs., Eli Lilly & Co., Indianapolis, IN 46282.

Cholecystokinin (CCK) has been proposed as an etiologic factor in anxiety, since the tetrapeptide form (CCK-4) induces panic attacks in humans and CCK antagonists have demonstrated anxiolytic-like effects in animal models of anxiety. In this study, two structurally distinct series of potent, selective antagonists for the brain CCK receptor (CCK-B) were assessed for anticonflict effects in a primate model of anxiety. Lever press responses of squirrel monkeys were maintained under a two-component schedule. During the first component, a response during the 3-min interval elapsed; during the second component, every 30th response during the 3-min interval resulted in the delivery of a mild electric shock (punishment or conflict procedure). At 0.3-10 mg/kg, p.o., the CCK-B antagonists LY 262691 and LY 262684 (pyrazoline class) all increased punished responding, with peak increases reaching approximately 150% of control performance levels at the 1.0 to 3.0 mg/kg doses. These doses had little effect on unpunished responding occurring in the alternate component. Agents with clinical efficacy in anxiety have been shown widely to produce increases in punished responding in this type of model. These results suggest that CCK-B antagonists of diverse structural types may be useful in the treatment of anxiety. The lack of suppression of either component further suggests that such treatment could be accomplished without accompanying sedative activity.

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We reported that feeding to neuropeptide Y (NPY) is reduced in anorectic tumor-bearing (TB) rats (Ann. NY Acad. Sci., 611:497,1990) and that hyperammonemia may be a primary cause of experimental cancer anorexia (Brain Res. 486:316,1989). In this study we tested the effect of hyperammonemia on NPY-induced feeding in normal rats. Two weeks after the implantation of cannulae into the periventricular hypothalamic area, the external jugular vein of adult, male, F 344 rats was cannulated. Normal saline (SAL) was infused (2 ml/hr) for four days, after which half of these rats were switched to ammonia (ANN; 0.2-0.3 M ammonium acetate + ammonium bicarbonate (pH = 7.7). On the 4th day of ammonia infusion, noninfused (n=5), saline-infused (n=5) and ammonia-infused rats (n=7) were tested for feeding to 1 ug NPY. Compared to saline-infused rats, food intake was reduced by 65% (1 hr) and 58% (4 hr) in ammonia-infused rats. Although 0.2 M ammonium salts had no significant effect on twenty-four hr feeding, infusing 0.3 M ammonium reduced ad lib. food intake by 44%. These results indicate that hyperammonemia reduces NPY-induced feeding and suggest that the increase in blood ammonia concentration may be responsible for decreased feeding to NPY in TB rats.

Supported by Dept. of Veterans Affairs and PHS R01CA 68057.
421.5

ANIMAL MODEL OF ALZHEIMER'S DISEASE: IMPLICATIONS FOR SPATIAL DISORIENTATION, MEMORY LOSS AND WANDERING BEHAVIOR. D. Hoffman 1, & J. F. Ryan. Department of Psychology, State University of New York at Plattsburgh, Plattsburgh, NY 12901

Spatial information from cortical areas is received and processed by the dentate gyrus and hippocampus. We have found that degeneration of this area in rats results in spatial memory impairments and mimics the wandering behavior in Alzheimer's disease. The present study measured spatial memory impairments using the Morris water maze task. A baseline of maze performance was recorded for twenty-two Long-Evans hooded rats for a period of six days (36 trials). The experimental group received bilateral intradentate injections of colchicine (15 μg), while a sham group received bilateral intradentate injections of saline. Ten days following surgery, both groups were given a five-trial probe trial to the maze for a period of three days (18 trials). By changing the platform location of the maze (i.e. the goal) a new task was introduced and tested for three days (18 trials). In comparison to the control group, the lesioned animals learned the behavior equally as well during baseline. However, they failed to recover their memory for the maze after surgery and also failed to learn a new task, whereas the control group consistently located the platform across all testing conditions. The results indicate that disruption to the spatial information processing system (dentate gyrus) impairs and impairs recently acquired spatial memory, while including new input of the spatial task. This suggests that in Alzheimer’s disease, spatial disorientation in familiar environments could be related to degeneration of the dentate gyrus. Furthermore, the introduction of a new environment may exacerbate an already disoriented condition.

421.6


Adult male rats received transplants (TP) of dissociated 30-day-old cultured cortical astrocytes (AST) into the nucleus basalis magnocellularis (NBM) or into the frontal and parietal cortex (CF) immediately after an intracerebral injection of ibotenic acid (BIS) into the NBM. Nine days after surgery rats with AST-TP into NBM or CF were as impaired in the acquisition of a passive avoidance (PA) task as untreated counterparts. No effects were found for PA retention. Brains were processed for BIS. GFAP-ICC, ChE activity and cytochrome C (CO) 14 days after TP. Host brain gliosis, but no surviving AST-TPs, were observed in the NBM. AST-TPs had no effect on size of the lesions. Concentrations of ibotenic acid used to lesion the NBM killed AST in vitro within 24 hours, suggesting a similar fate for AST-TPs in NBM. BIS-labeled AST were observed in NBM, but these AST-TPs produced severe lamine disruption, gliosis and decreased ChE and CO staining. Supported by ROI NS26685 and PO1NS21469.

421.7


In this study, characteristics of behavioral and physical disturbances of rats with ibotenic acid lesions of the bilateral nucleus basalis of Meynert (NBM) were analyzed in connection with physical disability or abnormal behavior in patients of Alzheimer's disease. Male Wistar rats (9 weeks old) were used. They were maintained in the air-conditioned room with 12:12 LD cycle, and housed in the running wheel or the activity meter (Santen SV-10, Toyo-sangyo) cages. Ibotenic acid (50 μg/0.5 μl) was injected into the NBM (Faxinos & Watson: A7.7,1.3,2.7,3.1,4,7) under pantobarbital anesthesia (50 mg/kg, i.p.). After the surgery, rats were forcibly given milk daily. In the results, the NBM-lesioned group revealed body weight loss, disruption of food-intake, and disturbance of circadian rhythm of activity with the increased daily activity time. These disturbances recovered within 1 week accompanying with recovery of body weight in most of the rats. However, several rats showed no recovery with urinary incontinence. The results suggest that these disturbances may be attributable to damage to the adjacent lateral hypothalamus, and are interested in the physical disability model.

421.8

MEMBRANE DEPOSITIONS IN A CELLULAR MODEL FOR ALZHEIMER'S AMYLOTOXICITY. G.E. Monste, R.E. Bylichka, B. Tera, F. Sejdi1 and F.A. Hulda. Massachusetts General Hospital, McLean Hospital and Harvard Medical School, Boston, MA 02114.

In this study, we compared the number and size of lipofuscin inclusions in the NBM of AD patients, including cell-cell interaction. Supported by NIH AG002162 and Metropolitan Life Foundation, OGM supported by University of Zulia.

421.9

Smile plaques do not support neurite outgrowth in vitro. M.K. Carpenter 1, K.A. Crutcher 2, S.B. Kater 1. 1 Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins. CO 80523; 2 Dept. of Neurosurgery, Univ. of State Univ., Fort Collins. CO 80523; 2 Dept. of Neurosurgery, Univ. of

Although senile plaques represent a consistent neuropathological feature in Alzheimer's brain, it is not known what role plaques play in the etiology of the disease. Both growth-promoting and growth-inhibiting influences have been shown to affect neuron survival and neurite outgrowth in vitro. We hypothesized that plaques might either promote or inhibit neurite outgrowth in vitro. Since plaque components consist of amyloid beta, the present study determined whether neurite outgrowth was affected by senile plaques in culture. This was accomplished using a novel technique in which the plaque components were extracted from living brain tissue using a high concentration of NaCl. Neurons growing on a substrate with a high concentration of NaCl were cultured on plaque extracts for 72 hours, and then stained with a fluorescent dye (the acridine orange) that stains live cells red, and dead cells blue. The results indicated that neurite outgrowth was not significantly affected by plaque extracts. However, neurites that extend from neurons adjacent to plaques appear to turn away from the plaques. Supported by RO1NS26685 and PO1NS21469.

421.10

QUISQUALATE INJECTION INTO THE NUCLEUS BASALIS MAGNOCELLULARIS INDUCES SEIZURE-RELATED BRAIN DAMAGE. D. Price 1, D. F. Wozniak 2, S. Sue 3, and J. W. O'Leary. Washington Univ, St. Louis, MO 63110.

Dopamine (DA), quisqualate (Q) and N-methyl-DL-aspartate (NMA), are excitotoxins that have been used to destroy neurons in the nucleus basalis magnocellularis (NBM) of adult rats. NBM lesions produced by intra-NBM injections of Q or NMA are associated with reliable seizure activity. This is consistent with the hypothesis that cholinergic neurons play an important role in memory and that loss of these neurons may contribute to memory impairment in Alzheimer's disease. Here we report the effects of intranuclear quisqualate administration on DA, NMA and glutamate receptor-induced seizures in rats. In the present study, we used a similar lesion model, including cell-cell interaction. Supported by NIH AG012162 and Metropolitan Life Foundation, OGM supported by University of Zulia.

421.11

Alzheimer's disease (AD) is characterized by the extracellular accumulation of the amyloid beta protein. The pathogenesis of AD is still unclear. It has been suggested that the formation of amyloid beta peptide (Aβ) is related to the extracellular accumulation of Aβ. However, the mechanism of Aβ accumulation is not well understood. In this study, we investigated the relationship between Aβ accumulation and the cellular localization of APP in AD brains. The results indicate that Aβ accumulation is associated with the cellular localization of APP. Supported by NIH NS26685, MRC supported by NIH NS02075.

Various excitotoxic agents, including ibotenic (Ibo), quisqualic (Qss) and N-methyl-D-aspartate (NMA), have been used as lesioning agents to destroy cholinergic nuclear basalis magnocellularis neurones in adult rats. Destruction of these neurones by Ibo resulted in rats with similar learning/memory deficits. This is consistent with the hypothesis that cholinergic neurones play an important role in the establishment of the learning/memory deficits in Alzheimer's disease (AD). However, recent reports that Qss decreases at least as many basal forebrain cholinergic (BFC) neurones as Ibo or NMA, without producing consistent learning/memory deficits, raises the important question whether other factor than BFC neuronal loss might account for the impairment. It is not known whether injection of excitotoxins into the basal forebrain destroys somatostatinergic (SOM) as well as cholinergic neurones, but in the brains of patients with AD, both of these transmitter systems are reportedly affected. The present study was undertaken to determine what effect NMA has on basal forebrain SOM (BFS) neurones. Injection of NMA into the basal forebrain caused a lesion which, by routine histopathological evaluation, appeared well confined to the injection site. The largest and most conspicuous group of BFC neurones are those in the horizontal/horopter nuclei, as well as the Caudate nucleus. However, in nearby portions of caudate nucleus there was a 30% loss of SOM neurones with no loss of cholinergic neurones. Our findings signify the following: 1) BFS neurones are extremely sensitive to excitotoxicity-induced degeneration; 2) An excitotoxic mechanism could account for loss of either BFS or BFC neurones in AD; 3) The learning/memory deficits associated with an BFS loss, as with AD, could be due in part, to loss of BFC neurones. Supported by NIH grants AG05681 (DFW & JWO) and RSA MHE3894 (JWO).


Previously, we have reported that bilateral radio-frequency lesions of the nBM resulted in deficits in differential conditioning performance. As compared to control animals, rats with bilateral nBM lesions showed impaired discrimination (i.e., reduced scores, increased trials required to reach criterion) on a bar-press task. Lesioned and control animals did not differ with respect to overall rate of responding. Results suggested that nBM-lesioned animals had an impaired ability to attend to and use stimulus contexts as guiding behavior. In the current study, we now report that such deficits persist months after lesions are induced. Twelve weeks post-lesion, ten months after their last exposure to the task, animals were retested. In order to model this experimental manipulation, initially, both lesioned and control animals showed poor discrimination, but on successive days, control animals improved whereas lesioned animals showed consistently poor discrimination ability. Supported by Sigma Xi Scientific Research Society Grant-in-Aid-of-Research to J.E.D. and A.E.B., and by UNM RAC grant 1-02996 to G.K.H.

421.13 INDUCTION OF THE BETA AMYLOID PRECURSOR PROTEIN IN THE SUBCORTICALLY LESIONED RAT CORTEX. V. Haroutunian, V. Bragin*, K. Davis, W. Wallace; Dept. Psychiatry and Center for Neurobiology, Mt. Sinai School of Medicine, New York.

We have reported that NMDA lesions of the rat nucleus basalis of Meynert result in the increased synthesis of beta-amyloid precursor protein (B-APP) in the cortex. In order to determine the normal physiology of function of B-APP in the intact brain, we have further characterized this induction. This induction is common to other cortically projecting neurotransmitter systems. Lesions of the dorsal raphe nuclei, which targets serotonergic innervation and ascending noradrenergic bundle, which targets adrenergic innervation, similarly induce the cortical B-APP. Thus, the induction of B-APP appears to be a general response of the lesioned cortex. However, treatment of naive rats with the acetylcholinesterase inhibitor, physostigmine does not alter B-APP synthesis, showing that increased levels of B-APP are not due to an increase in acetylcholine. The elevated B-APP synthesis is accompanied by increased B-APP mRNA indicating increased gene transcription. The induction occurs as early as one hour after lesion and persists for at least seven days. These observations suggest that B-APP may be interacting with other neurotrophic factors in the cortex in response to the loss of subcortical innervation.


Alzheimer disease (AD) is initially characterized by reduced CBF, spatial memory dysfunction, increased tempo of senile plaque deposition (SPD), atrophy of spatially targeted tangles (NT), glutathione, acidic protein (GFAP) and by hippocampal cell loss. β: NMR spectroscopy and immunohistochemistry were used to measure β-amyloid precursor protein (B-APP) elevation which indicates an increase in membrane phospholipid synthesis. We subjected aged rats to cerebrovascular insufficiency (CVI) for 9 weeks as we have described (Brain Res, 426, 1988). At 9 weeks after CVI, we measured CBF and brain weights for spatial memory, β: NMR spectroscopy/imaging using 1H localization for the hippocampus. Potentially, B-APP elevation was examined for CA1 sector cell counts and GFAP reaction. Results show that CBF rats but not matched controls developed spatial memory impairment in the Morris Water Maze task and elevation of B-APP and β-amyloid. β: NMR spectroscopy and immunohistochemistry. In addition, aged CBF rats had significant cell damage to CA1 and increased GFAP immunoreaction throughout the hippocampus. Rats had cortical and hippocampal CBF reduction 3 weeks after chronic ischemia but only hippocampal blood flow remained low for 9 weeks after CVI (equivalent to 4-6 human years).

These results suggest that membrane phospholipid synthesis is increased in CVI rat hippocampus possibly due to proliferation of reactive GFAP-positive astrocytes. We concluded that chronic CVI in aged rats induce a syndrome that mimics the cognitive, metabolic and morphologic changes seen in AD. It is tempting to speculate that the pathologic deficits seen in the early course of AD could also be triggered by chronic CVI.

Funded by the Ontario Mental Health Foundation


Patients suffering from Alzheimer's Disease (AD) display a number of abnormalities in circadian rhythms that may alter normal circadian rhythms in AD. AD could be due, in part, to loss of BFS neurons. Supported by NIH grants AG05681 (DFW & JWO) and RSA MHE3894 (JWO).

421.16 ATROPHY OF BASAL FOREBRAIN CHOLINERGIC NEURONS IS MODELED BY MOUSE TRISOMY 16 TRANSPANT. D.M. Holtzman, V.L. St.J. DeArmond*, P.H. Gage, C. Jenkins* and M. Mohly. Dept. of Neurology, Pediatrics, and Biochemistry, UCSF, San Francisco, CA 94143 and Dept. of Neurosciences, UCSF, La Jolla, CA 92033.

Vulnerability of specific brain regions and neuronal populations is a characteristic feature of Alzheimer's disease (AD) and Down syndrome (DS). One of the vulnerable populations includes cholinergic neurons in the basal forebrain. We transplanted brain tissue from mouse trisomy 16 (T16), an animal model of DS, in order to develop a model to study the neuronal degeneration which occurs in AD and DS. Suspensions of fetal T16 or control septum were injected into hippocampus of young adult mice. At one month post-transplantation, there was no neuronal survival and near-total growth from all grafts. However, after six months, T16 cholinergic neurons and their proximal neurites frequently appeared shrunken and atrophic. They were significantly smaller than controls (153.9 vs. 166.2 μm). The atrophy of cholinergic neurons was selective in that significant difference in weight was seen in the size of non-cholinergic neurons; controls remained larger than T16. Our findings indicate that one can model a genetic disorder leading to cholinergic atrophy. It should allow for potential therapies to prevent genetically determined neurodegeneration.

Orvig and coworkers (J.Amer.Chem.Soc., 108:5033, 1986) have shown the aluminum-related neurotoxicity can be increased by an order of magnitude by administering aluminum as aluminum maltol. Following this lead we have prepared a lipophilic complex, tri(3-hydroxyflavonato)aluminum (aluminum flavonol, AIFl3), and have demonstrated acute and chronic neurotoxic effects in rabbits when administered intravenously as a single injection. AIFl3 is not water soluble but a stable suspension can be prepared using a Brinkman tissue homogenizer. Intraventricular injection of 0.1mL of a 0.5% suspension in New Zealand albino rabbits produced seizures at 18 days, and SMI-31 (monoclonal antibody to phosphorylated medium and heavy neurofilament proteins) immunoreactivity (ir) revealed extensive neurofibrillary degeneration in cortical layers III and V. Additional animals remained asymptomatic after four weeks but had numerous SMI-31 ir neurons in the neocortex and subiculum. Animals exposed to 0.1mL of 7.5mM AIFl3 showed only subtle symptomatology after five months. These animals also demonstrated neocortical neurofilamentous tangles. An interesting property of AIFl3 is its autofluorescence, and using fluorescence microscopy we have observed particles of the compound in macrophages within the ventricles. AIFl3 and other novel aluminum chelate complexes represent important new tools for developing animal models of chronic aluminum neurotoxicity. Supported by the American Health Assistance Foundation.


In vitro assays were used to monitor and compare the kinetic behavior of microtubule assembly induced by tau proteins isolated from Alzheimer’s disease (AD) and non-demented age matched control brains. Tau from 4 of 5 AD cases induced slower assembly and a steady state assembly approximately 50% of that stimulated by tau from 3 control cases. Tau from the most severe cases was least effective at promoting assembly. Dark field microscopy of the control samples indicated abundant microtubule formation and many microtubule bundles. Fewer microtubules were observed in the AD samples and bundling could not be detected. There was no obvious difference in the ultrastructure of microtubules formed in both cases. SDS-PAGE analysis revealed lower amounts of tau in microtubule pellets from experimental compared to control cells which supports the assembly data. AD tau is modified by phosphorylation (PNAS, 83:4040-4043; 4913-4917). These results indicate that tau modification compromises its function in helping provide a population of stable neuronal microtubules needed for critical functions such as axonal transport. AG 06383, NS 17731.


The relationship between tau and Alz-50 was studied by immunofluorescence in HCN-1A, and N1E-115, B103, and the F3 variant of PC12 cells. Results were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Neurites</th>
<th>Alz-50</th>
<th>Tau</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCN-1A (D)</td>
<td>Yes</td>
<td>Negative</td>
<td>+ all neurites &amp; soma</td>
</tr>
<tr>
<td>HCN-1A (U)</td>
<td>No</td>
<td>Negative</td>
<td>Very weak</td>
</tr>
<tr>
<td>N1E-115</td>
<td>Yes</td>
<td>+ all cells</td>
<td>+ all neurites &amp; soma</td>
</tr>
<tr>
<td>B103</td>
<td>Yes</td>
<td>+ all cells</td>
<td>+ all neurites &amp; soma</td>
</tr>
<tr>
<td>F3</td>
<td>No</td>
<td>Weak</td>
<td>+ all cells</td>
</tr>
</tbody>
</table>

In some cell lines, Alz-50 was present in all the cells, suggesting that Alz-50 is not a marker of cell death. The difference between the expression of Alz-50 in B103 and F3 cell lines is interesting when considered with the fact that almost all of the B103 cells constitutively express neurites in vitro, whereas F3 cells rarely express neurites. This interesting contrast is consistent with the hypothesis that Alz-50 may be associated with the process of neurogenesis. These cell lines may be a valuable source for further understanding the role of Alz-50 in aging and normal development.

BRAIN TAU LEVELS ARE ELEVATED IN ALZHEIMER’S DISEASE. S. Khatoon*, L. Grunke-Iqbal* and K. Iqbal, NYS Institute for Basic Research, Staten Island, N.Y. 10314.

Microtubule associated protein tau which stimulates the assembly of αβ tubulin heterodimers into microtubules is abnormally phosphorylated in Alzheimer disease (AD) brain, and is the major component of paired helical filaments. In the present study, the levels of tau, abnormally phosphorylated tau and of tubulin were determined in brain homogenates of AD and age matched normal cases. A radioimmuno slot blot assay was developed using a primary monoclonal antibody (mAb), Tau-1 and anti-mouse IgG as secondary antibody. For assaying the abnormally phosphorylated tau, the blots were treated with alkaline phosphatase prior to immunolabeling. Amounts of α and β tubulin were determined by an ELISA using mAb YL1/2 to α and mAb KMX-1 to β tubulin. The levels of total tau were about 6-fold higher in AD (5.9±1.3 ng/μg protein) than in control (1.0±0.2) cases. Most of the tau in AD brain was abnormally phosphorylated (4.3 ng/μg protein). In contrast there were no significant changes in the levels of tubulin in AD brain. These studies show (1) that there is a marked increase in brain levels of tau in AD, and (2) that this increase is due to the accumulation of abnormally phosphorylated tau in the affected tissue. (Support by NIH grants AG05892, AG08076, NS18105 and AG04220 and a grant from AHA).
In Alzheimer's disease (AD), neurofilbrillary tangles (NFT) and dystrophic neurites contain paired helical filaments (PHF) composed largely of abnormally phosphorylated tau, a microtubule-associated protein. The kinases that phosphorylate tau in NFT are not yet known. Casein kinase II (CK-II), a highly conserved serine/threonine kinase, phosphorylates tau in vitro. NFT extract is immunoprecipitated, and purified CK-II can bind NFT on AD brain sections. We explored the nature of this binding using purified CK-II and purified PHF. To detect any PHF-associated CK-II, PHF was purified by a ammonium sulphate and Davis. Immunobots of PHF showed no CK-II, indicating either that the amount of CK-II present was too little to detect, CK-II does not bind PHF, or that in the PHF purification procedure CK-II could bind PHF. CK-II was added to centrifuge tubes containing PHF, spun to pellet the PHF, and the amount of CK-II pelleting with the PHF was measured on immunobots. Although some CK-II, which can form filaments in vitro, pellets in control tubes, more CK-II pellets in PHF tubes. Low salt enhances co-pelleting, suggesting an ionic interaction. Thus, CK-II binds PHF in vitro. In vivo, the normal functions of CK-II might be disrupted by its abnormal localization in AD. In addition, since the phosphorylation of a substrate by CK-II can be enhanced by previous phosphorylation of the substrate, CK-II might contribute to the overphosphorylation of tau in PHF.

Characterization of HMW Tau in the Peripheral and Central Nervous System: N. Teleman*, T. Sanberg, and M. Oliver, Dept. of Cell Biology & Anatomy, The Chicago Medical School, North Chicago, IL 60064

Tau is a complex family of neuron-specific microtubule-associated proteins that are encoded by a single gene. The major tau proteins in brain are a complex of low molecular weight (50-70 kDa). However, a HMW tau isoform (110 kDa) has been found in peripheral nervous tissue as well as in PC12 cells. Little is known about the distribution of this HMW tau form in the nervous system. Is this isoform restricted to the PNS, and are the properties of HMW tau different from those of other isoforms? In order to address this issue immunoblotting analysis was employed and such studies revealed that while LMW tau was present throughout CNS samples, HMW tau was largely restricted to tissues that were obtained from the PNS. An intermediate-sized tau isoform (MMW tau, 90-110 kDa), was also identified in this study. This isoform was localized in the optic nerve (ON) as well as the cerebellum. In the ON, some of the molecular properties of HMW tau, was examined its behavior under a series of conditions, namely its gel mobility after treatment with phosphatase, its heat stability and its partitioning when exposed to cold-calcium and Triton extraction paradigms. Interestingly, HMW tau partitioned with the soluble fraction while LMW & MMW tau partitioned mainly with the stable microtubule pellets. Immunocytochemical studies revealed that DRG cell bodies stain with tau specific antibodies. This is in sharp contrast to previous studies that indicated that neuronal cell bodies in brain don't exhibit staining with tau-1 antibody. Immunobots from immunoblot of DRG contain only the HMW tau form we can suggest that the DRG cell bodies contain HMW tau and that the tau-1 epitope is not masked in these cell bodies. Northern blot analysis of total RNA from different regions of the adult nervous system revealed different sized tau mRNA's (6-8 kb). This suggests that alternative splicing of tau mRNA is a significant factor in generating tau heterogeneity. The HMW tau generated by such a mechanism has unique characteristics from other tau forms.

Time-Dependent Effects of Intrahippocampal NMDA on the Microtubule Proteins Tau and MAP2 in PC12 Cells. R. K. Ruhl* and K. K. Harris, S.H. Aschner* and F. Yamamoto.

422.7
e-Dependent Effects of Intrahippocampal NMDA on the Microtubule Proteins Tau and MAP2

In this study, we have examined three other candidate proteins, vimentin, the low molecular weight neurofilament (NF-L), and β-tubulin. RNA prevalence in AD and control sections from each region was measured by a solution hybridization method (RNase-protection assay), carried out in triplicate using six concentrations of total RNA per sample. The mass of each specific message per unit mass of total RNA was calculated (pg/μg).

<table>
<thead>
<tr>
<th>Marker</th>
<th>TCCX (C)</th>
<th>TCCX (AD)</th>
<th>CRL (C)</th>
<th>CRL (AD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAP</td>
<td>4.7±0.5</td>
<td>29.8±7.2</td>
<td>11.7±1.4</td>
<td>8.2±0.8</td>
</tr>
<tr>
<td>Vimentin</td>
<td>1.6±0.1</td>
<td>2.6±0.6</td>
<td>0.8±0.1</td>
<td>0.68±0.07</td>
</tr>
<tr>
<td>NF-L</td>
<td>2.0±0.04</td>
<td>1.9±0.03</td>
<td>0.8±0.1</td>
<td>0.56±0.05</td>
</tr>
<tr>
<td>β-Tubulin</td>
<td>4.3±0.1</td>
<td>3.5±0.4</td>
<td>3.8±0.4</td>
<td>4.1±0.4</td>
</tr>
</tbody>
</table>

The 4.5 fold increase in GFAP message between AD and control cortex was the only statistically significant change. Two cortical AD samples had 4 fold elevations of vimentin RNA suggesting microglial/macrophage proliferation, but other samples were within the control range. No change in NF-L or β-tubulin were detected, although northern analysis suggested a different size for the NF-L mRNA in AD T-CX. As expected, no changes were observed in the relatively intact AD cerebellum. We thank SA Johnson for organizing the brain tissue samples and CA Zavag for modifying the NF-L clone. Supported by AG07892, AG00093, AHA-GIA 891079, and AHA-EIA 900713.

Morphometric studies have suggested that dendritic plasticity is altered in Alzheimer's disease. MAP-2, a microtubule associated protein, has been shown to be a dendritic specific protein whose levels may parallel the volume of the dendritic compartment. Thus, measurement of MAP-2 mRNA levels could reflect the state of the dendritic compartment. Total RNA was extracted from AD and control temporal cortex. MAP-2 mRNA content was measured by oligo-dT slot hybridization. Data analysis demonstrated that there was a 57% decrease in MAP-2 messages in AD when compared to age-matched controls. Area 4, primary motor cortex, has not previously been thought to be significantly affected in AD. (Supported by R35 AG 09016, P01 AG 03644, R01 AG 01221, P30 AG 08665 (PDC) and ADRDA PRG-89-120 (KERR).)


The neuronal cell contains subpopulations of microtubules (MT) as defined by their composition and function. Different MAPs, and their isoforms are associated with different MT populations. In addition, these different MAPs show selective sensitivities to different MT depolymerizing conditions. In order to investigate the early events in MT degeneration, we have used 3T3 cells to examine the effects of intracellularly injected hippocampus. The cells were transfected with human tau isoforms. Northern analysis revealed that tau protein levels in the transfected cells were similar to those in normal rat brain. Expression of individual tau isoforms confers unique cellular morphologies as well as different MT stabilities to depolymerizing conditions. These morphological differences are further accentuated by the MT depolymerizing conditions of cold and calcium. Degradation of MTs resulting from various treatments shows that the MT subpopulations differ from each other. These observations, however, an intracellular MT matrix remains intact in the cell body which contains concentrated "pools" of tubulin. In contrast, cold treatment causes a progressive degradation of MT into small fibrils without altering the cellular morphology. Increased levels of intracellular calcium causes a redistribution of the MT networks into bundles yet does not change the cell's morphology. The different cytoskeletal changes caused by these MT depolymerizing conditions, as well as the morphological changes caused by expression of different tau isoforms, reveals that there are numerous subpopulations of MTs. We will be focusing on the effects of elevated intracellular levels of calcium as it relates to cell morphology, cytoskeleton, and organelle redistribution, and the calcium regulated phosphorylation events of tau.

Expression of tau protein in non-neuronal cells results in the redistribution of the microtubule (MT) cytoskeleton into thick bundles of tubulin-containing MTs (Lewis et al., 1988). The molecular mechanism by which tau- and tubulin bundling of MTs is not understood, therefore experiments were performed to reconstitute MT bundling using purified bovine tubulin and recombinant human tau isoforms. Tubulin was assembled into MTs by incubating with taxol in assembly buffer. The MTs were isolated by centrifugation, mixed with various concentrations of T4 (tau isoform containing four MT binding domains), and examined by negative stain electron microscopy. In the absence of tau the MTs were randomly distributed. When MTs were incubated with T4 and tubulin, molar ratios of 1.0:0.9 the MTs appeared as loosely- and tightly-packed parallel arrays. MTs assembled into MTs condensed into thick bundles containing multiple layers of tightly packed parallel arrays. MT bundling was observed with porcine tau and other human tau isoforms, and was blocked by the addition of 0.5M NaCl or 0.3M tris(2-carboxyethyl) phosphine. A tau deletion construct that contained only the four MT binding domains plus 19 amino acids to the C-terminus was capable of bundling MTs. These data further refine the region of tau required for MT bundling in vitro and demonstrate that bundling can be reconstituted using only tubulin monomers and tau protein.


Individual human tau isoforms purified from a E.coli system expressing each specific clone (Goedert and Jakes, 1990) were used in this study. Of the six isoforms cloned, one used in this study was tau with either one or four microtubule-binding repeats in the carboxy-terminal half of the molecule (T3R, T4R) and a four repeat tau with the 58 amino acid insert in the amino-terminal region of the molecule (T4RL). The isoforms were phosphorylated by cAMP-dependent protein kinase (cAMP-PK) and Ca++/calmodulin-dependent protein kinase II (CamKII). The phosphorylation was subject to SDS-PAGE, gel electrophoresis (1D PAGE), two-dimensional nonequivalence pH gradient electrophoresis (2D NEPHGE), 2D phosphopeptide mapping, calcium hydrolysis and a binding assay. Phosphorylation by either kinase resulted in the migration of all three isoforms on 1D PAGE. cAMP-PK produced two slower migrating species, the slower of which aligned with the single band produced by phosphorylating with CamKII. 2D NEPHGE revealed that T4R exalted as one isoform, while T3R and T4RL appeared to exist as 5 and 7 isovariants, respectively. Phosphorylation also increased the microtuboreactivity of the isoforms on 2D NEPHGE. The 2D phosphopeptide maps demonstrated that the isoforms were all phosphorylated in a similar manner, with the two kinases producing distinct peptide maps. The three tau isoforms were all susceptible to calcium hydrolysis, however T3R was proteolyzed at a slower rate than either T4R or T4RL. The hydrolysis of the isoforms by calpain was significantly increased after phosphorylation by CamKII. Phosphorylation by cAMP-PK did not alter calpain-induced proteolysis. In addition, phosphorylation appears to modulate the binding of the tau isoforms to tubulin. Supported by NIH grants NS27538 and AG00659 and a grant from the American Health Assistance Foundation.


The phosphorylation state of tau has been postulated to modulate its interactions with other cytoskeletal components and also may regulate its susceptibility to proteolysis. We are examining these processes in a system based on recombinant tau protein which is an important component of paired helical filaments (PHFs), which are found in Alzheimer's disease. In this study we examined the in vitro phosphorylation of bovine tau by cAMP-dependent protein kinase (cAMP-PK), Ca++/calmodulin-dependent protein kinase II (CamKII), and protein kinase C (PKC), and how phosphorylation by these kinases modulates its hydrolysis by calpain or binding to microtubules. Phosphorylation by tau kinases also altered the binding of tau to microtubules. These results suggest a role for cAMP-PK phosphorylation in regulating the degradation of tau. Abnormal phosphorylation could result in a protease-resistant tau population which may contribute to the formation of PHFs in Alzheimer's disease.

Supported by NIH grants NS27538 and AG00659 and a grant from the American Health Assistance Foundation.

ASSOCIATION OF TAU(+) WITH RIBOSOMES IN ALZHEIMER'S DISEASE. S. Ch. Papasozomenos and Y. Su*. Univ. of Texas Med. Sch., Houston, TX 77025.

We showed that in Alzheimer's disease (AD) immunoreactivity is associated not only with the abnormal filament but also with ribosomes (Lab. Invest. 60:123, 1989 and 60:375, 1989). We also observed that in chromatolytic Pick's cells and in Pick's bodies immunoreactivity is associated with ribosomes. In this study, we have isolated ribosomes from six AD cases and controls and used four monoclonal anti-ribo antibodies (Tau-1, Tau-2, Tau-5 and Tau-4). Immunoblots analysis and EM immunogold labeling to further investigate the association of + with ribosomes. In immunoblots of SDS extracts, three + polypeptides of 60-, 62- and 66-kDa were present but only in regions that in parallel histologic preparations contained + immunoreactivity. De-phosphorylation was necessary to demonstrate these three + polypeptides with Tau-1 and enhanced staining with Tau-5. Also, the ribosomal 62- and 66-kDa + polypeptides co-electrophoresed with the most slowly moving polypeptides in SDS extracts of cortical homogenates from the same region. Controls were negative. In addition, all four antibodies decorated isolated ribosomes at the EM level. The purity of the preparations was verified by EM of the ribosomal pellet and negative staining with uranyl acetate of ribosomal solutions. These findings show that AD is indeed associated with ribosomes and will further elucidate the etiopathogenesis of AD.
ALZHEIMER'S DISEASE: CYTOSKELETON

422.17 SPECTRIN (240/235E) AND DENDRITIC REORGANIZATION IN ALZHEIMER'S DISEASE. Angela Lee and Neil W. Kowall. Neurology Service, Massachusetts General Hospital, Boston MA 02114

Neuronal degeneration in Alzheimer's disease (AD) is associated with axonal and dendritic plasticity and reorganization. The axonal microtubule-associated protein tau is also localized to the somatodendritic compartment of degenerating neurons in the form of neurofibrillary tangles (NFT) and dystrophic neurites. It is not known if a more generalized disruption of neuronal polarity occurs in AD. In normal neurons, subtypes of the major submembranous cytoskeletal protein spectrin are differentially distributed in axons and dendrites suggesting a possible role in the generation and maintenance of neuronal polarity. We examined the distribution of dendritic spectrin in normal and AD hippocampi with a monoclonal antibody (1G9) that recognizes the transmembrane and dendritic isoform of spectrin (240/235E). We sought to determine whether specific alteration of the somatodendritic compartment occurs in AD. In normal human hippocampus, red blood cells were prominently stained. A higher band of immunoreactivity defined the dentate gyrus granule cells and the inner molecular layer. Neurons were prominent in CA1 with occasionally intense somatodendritic immunoreactivity in the dentate gyrus extended into the outer molecular layer and a band of decreased immunoreactivity was seen in the polymorph layer. Occasional intensely reactive neurons containing NFT or GVD were found in CA1 and the subiculum. Spectrin (240/235E) retains its normal somatodendritic localization in the dentate gyrus in AD but its distribution shifts in conjunction with dendritic reorganization. GVD contain prominent dendritic spectrin immunoreactivity. A small number of NFT-bearing neurons are intensely immunoreactive suggesting that the synthesis and/or posttranslational modifications of dendritic spectrin may be altered in AD.

ALZHEIMER'S DISEASE: NEUROCHEMISTRY II

423.1 ALTERATIONS IN THE PHYSICAL PROPERTIES OF BRAIN MEMBRANES IN ALZHEIMER'S DISEASE. A. S. Bloom, F.G. Antuono and L.F. Staat. Department of Pharmacology and Neurology, Medical College of Wisconsin, Milwaukee, WI 53226

Several studies have indicated that there are changes in the fluidity of a variety of biological membranes during aging and in Alzheimer's disease (AD). We have expanded upon these observations by examining differences between the fluidity of purified synaptic plasma membranes (SPM), prepared from the brains of persons dying with AD (n=11) and aged-matched controls (n=6). Membrane fluidity was determined in 7 brain regions using fluorescence polarization. A temperature of 35 C was used. When diphenylhexatriene (DPH) was used as a fluorescent probe, (it measures membrane phospholipid ordering), decreases in polarization were noted for all regions compared to normal SPM prepared from frontal and parietal cortex of AD brains when compared to controls. Polarization values decreased from 0.2373 to 0.2038 in parietal cortex and from 0.2797 to 0.2630 in temporal cortex. Significant changes were not seen in the other regions studied. When TMA-DPH which localizes to the more superficial regions of the rod photoreceptor bilayer was used as a probe, decreases were noted in polarization of occipital cortex and hippocampus of AD brains. Polarization values decreased from 0.3487 to 0.3364 in the hippocampus and from 0.3414 to 0.3340 in temporal cortex. Again, significant changes were not seen in other regions. Lastly, the effects on the polarization of 12-anhydroxystearic acid (12-A5) fluorescence, which reports on the mobility of phospholipid acyl chains, were examined. Significant differences were seen only in the cerebellum, where polarization was increased from 0.3136 to 0.3429 in AD brains. These data indicate that there are regionally specific changes in synaptic plasma membrane ordering in AD brains that correlate with areas where AD pathology is observed. It is yet to be determined if these alterations are due to changes in membrane lipid or protein composition.

423.2 INCREASED SATURATION OF FATTY ACIDS IN ALZHEIMER'S DISEASE. P.G. Antuono, G. D. Chervitz* and K. C. Ho'. Departments of Neurology and Pathology, Medical College of Wisconsin, Milwaukee, WI 53226

Compositions of phosphatidylethanolamine (PE), lysophosphatidylethanolamine (lyPE) and phosphatidylcholine (PC) fatty acids (FA) were determined in post-mortem brain tissue of 12 AD patients and 8 controls. The two groups were matched for several clinical and post-mortem variables. PC, lyPE and PE were transmethylated and the resulting FA methyl esters were analyzed by gas liquid chromatography. FA compositions from white and gray matter of the frontal and occipital lobes were similar in two groups. The temporal cortex showed a trend towards a reduction in the ratio of monounsaturated to polyunsaturated FA, reaching a significant difference for arachidonic acid (20:4) in PC and PE. In the temporal lobe white matter showed a similar abnormality with a reduction of monounsaturated FA in PC and PE. Conversely polyunsaturated FA as docosahexaenoic acid (22:6n-3) were significantly increased in PC, lyPE and PE; arachidonic acid was increased in PC.

These results suggest a regional decrease in monounsaturated FA and increase in polyunsaturated FA in the temporal lobe of Alzheimer patients. Although this change may be secondary to the pathological changes of this disease, it may contribute to the alterations in membrane fluidity and neurotransmission reported in AD.


The multicatalytic protease (proteasome) and the calpains are two cytoplasmic, non-lysosomal protein-degrading systems present in many cell types. We have begun to evaluate the involvement of these systems in protein turnover in neurons. Protease activity was monitored in cerebellar granule neurons grown in primary culture by use of membrane-permeable fluorogenic substrates, such as Suc-LLVY-AMC. Experiments with purified proteases confirmed that both calpain and the multicatalytic protease readily degrade the substrates. Granule neurons produced a time- and dose-dependent substrate hydrolysis. Protease activity occurred intraneuronally, and did not result from any enzymes secreted into the medium. Several observations argue that the activity is due to the multicatalytic protease. A protease with the size (700 kD) and properties of the multicatalytic protease could be extracted and purified from granule neuron cultures. Moreover, the purified enzyme degraded the same substrates that were degraded by the granule neurons, and was selectively inhibited by the same agents which blocked the activity within the living neurons. The substrates cannot be ubiquitinated, because of a blocked N-terminus, indicating that the multicatalytic protease of neurons can degrade substrates without prior ubiquitination. Activation of calpain, evoked by raising the intraneuronal calcium content, caused only a small increase in substrate hydrolysis. The results indicate that the multicatalytic protease is a major cytoplasmic proteolytic system within neurons. The ability to monitor the activity of this protease in living neurons should facilitate identification of the factors that regulate its activity.
MULTICATALYTIC PROTEINASE COMPLEX, A NONLYSOSOMAL PROTEINASE, IS PRESENT IN SIGNIFICANT AMOUNTS IN A NEURONAL CELL LINE M.E. Pereira

Recent findings suggest that Alzheimer's disease (AD) may result from abnormal processing of multiple proteins. The multicatalytic proteinase complex (MPC) constitutes a major nonlysosomal proteinase with unique catalytic properties, splitting peptide bonds on the carboxyl side of hydrophobic (chymotrypsin-like activity), basic (trypsin-like activity) and acidic (peptidyl glutamyl activity) amino acids. Furthermore, MPC has been suggested to be the "catalytic core" of the ubiquitin/ATP-dependent protein degradation pathway. The neuropathological finding of ubiquitinated proteins in neurofibrillary tangles in AD and in other neuronal inclusion bodies may implicate MPC malfunction in neurodegenerative disorders, resulting in abnormal accumulation of ubiquitin-protein conjugates. In addition, MPC has chymotrypsin-like activity, the proteolytic activity believed to produce β-protein, the most abundant insoluble protein of senile plaques in AD brains. It is thus important to find a model system to study the regulation of MPC activity. We report that the cytosolic fraction of a neuronal cell line (HT4) has chymotrypsin-like, trypsin-like and peptidyl glutamyl activities, as shown by hydrolysis of the chromogenic substrates Cbz-Glu-7-AMC, Cbz-D-Ala-Leu-Arg-7-AMC and Cbz-Leu-Leu-Glu-7-AMC respectively. Specific immunoprecipitation of MPC with polyclonal antibodies generated in our laboratory resulted in the inhibition of the above described peptide activities, providing evidence for the involvement of MPC in these activities. Moreover we demonstrate that low concentrations of SDS (0.02%) stimulate the peptidyl glutamyl activity. The HT4 cells have been shown to differentiate with neuronal morphology, express neuronal antigens, synthesize and secrete nerve growth factor (NGF), and have receptors for NGF. Thus HT4 cells are an excellent tool for studying the regulation of MPC activity. (Supported by the Alzheimer's Association/New York City Chapter Pilot Research Grant PRG-90-114 to M.E.P.; NIH grant NS-17392 and an ADAMHA Research Scientist Award MH-00350 to S.W.; USPHS grant GM-34582 to S.I.R.)

SELECTION OF LOSSES OF SOMATOSTATIN SSI BINDING SITES IN FRONTAL AND TEMPORAL CORTEX OF ALZHEIMER'S PATIENTS S. Krajin, Y. Rabitschke, and R. Ouirion. Douglas Hospital Research Center, Dept. of Psychiatry, McGill University, Verdun, Quebec, Canada.

Somatostatin (SS) binding sites were found to be decreased in frontal and temporal cortices of Alzheimer's disease (AD) patients (Beal et al. 85). However, the human brain cortex was shown to contain two subtypes of SS receptors, SS1 (65-84)-selective (125-SS1, 2125-SS1, 423.8-SS1) and SS2 (423.9-SS1) with different distribution in AD brains without loss of C-peptide (Ann Nenl 22:2278, 1987; h:4:34, 1988). To determine whether the deficit is due to decreased levels of PMT mRNA we measured PMT mRNA using PCR amplification and densitometric quantitation of northern blots of PMT mRNA extracted from human C-peptide. Ten AD cases (75.6 ± 16 yrs; PM1 of 5.1 ± 1.3) were compared to 4 controls (71 ± 6 yrs; PM1 of 5.7 ± 1.1). There was no difference in C-1 PMT mRNA levels between the groups (AD:32.1 ± 14.8 units/μg RNA vs. controls: 40.6 ± 14.1). We conclude that decreased PMT protein in AD is not due to decreased transcription of PMT mRNA but may be due to either decreased translation of PMT mRNA or increased degradation of PMT protein.

SELECTIVE LOSSES OF SOMATOSTATIN SSI BINDING SITES IN FRONTAL AND TEMPORAL CORTEX OF ALZHEIMER'S PATIENTS S. Krajin, Y. Rabitschke, and R. Ouirion. Douglas Hospital Research Center, Dept. of Psychiatry, McGill University, Verdun, Quebec, Canada.

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OXYGEN-FREE RADICAL MEDIATED PROCESSES IN ALZHEIMER'S DISEASE. S.M. McIntosh, M.A. Trousil, D.J. Trousil, Department of Environmental Health Sciences, The Johns Hopkins University School of Public Health and Environmental Health Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 82105.

Free radical damage has been proposed as a factor in aging and in several neurodegenerative disorders, including Alzheimer's disease (AD). Changes of nucleic acids, proteins, and membranes may result from reactions with oxygen species, including superoxide and hydroxyl radical mediated peroxidative reactions. To address the role of free radicals in AD, we used chemiluminescence (CL), which measures bond breakage as it occurs, and the thiobarbituric acid-reactive substances (TBARS), which measure malondialdehyde (MDA) formed during lipid peroxidation. Tissue samples were obtained from the temporal cortices of six pairs of cases of AD and controls matched for age, postmortem delay, and accession date. In AD tissues, we found significant increases in CL (28%) and MDA formation (42%), as compared to controls. These differences were most apparent in basal levels from tissue supernatants that were not stimulated by iron, ascorbate, or hydrogen peroxide. Studies with biochemical probes to determine the predominant radical species in these cases are in progress. Our results support the hypothesis that oxidative stress may contribute to the damage and degeneration of neurons at risk in AD.

PHENYLETHANOLAMINE N-METHYLTTRANSFERASE (PNMT) activity and protein levels decrease in Alzheimer's disease (AD) brains without loss of C-1 neurons (Ann Nenl 22:2278, 1987; h:4:34, 1988). To determine whether the deficit is due to decreased levels of PMT mRNA we measured PMT mRNA using PCR amplification and densitometric quantitation of northern blots of PMT mRNA extracted from human C-peptide. Ten AD cases (75.6 ± 16 yrs; PM1 of 5.1 ± 1.3) were compared to 4 controls (71 ± 6 yrs; PM1 of 5.7 ± 1.1). There was no difference in C-1 PMT mRNA levels between the groups (AD:32.1 ± 14.8 units/μg RNA vs. controls: 40.6 ± 14.1). We conclude that decreased PMT protein in AD is not due to decreased transcription of PMT mRNA but may be due to either decreased translation of PMT mRNA or increased degradation of PMT protein.

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423.11 ALZHEIMER'S ANTIGEN: A RE-EXPRESSED PROTEIN OF IMMATURE CNS

423.13 A PILOT CROSSOVER STUDY OF THE ANGIOTENSIN CONVERTING ENZYME INHIBITOR (ACEI) SQ29,852 IN ALZHEIMER'S DISEASE.

423.14 INTERLEUKIN-1 INCREASES THE EXPRESSION OF AMYLOID PRECURSOR PROTEIN mRNA IN NEURONAL BUT NOT GLIAL CELLS.

423.15 COMPARISON OF THE EXPRESSION AND TOXICITY OF THE COOH TERMINAL 100 AMINO ACIDS OF APP CONTAINING A SIGNAL SEQUENCE.

423.16 DEXAMETHASONE AND DIBUTYRYL CYCLIC AMP INDUCE THE EXPRESSION OF ALPHA-1-ANTICHYMOTRYPSIN IN RAT ASTROCYTES: IMPLICATIONS FOR ALZHEIMER'S DISEASE.
423.17 IS ALZHEIMER'S DISEASE A LATE-ONSET, MOSAIC FORM OF DOWN SYNDROME? H. Potter and L. Geller. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115. Perhaps the most interesting clue to the cause of Alzheimer's disease is the fact that Down syndrome patients who live beyond the age of 30 or 40 develop dementia and neuropathology essentially indistinguishable from classic Alzheimer's disease. The implication of this finding is that trisomy for chromosome 21 is a sufficient cause of Down syndrome—is also capable of causing Alzheimer's disease, possibly through the overexpression of or dysregulation of genes residing on chromosome 21. A hypothesis has been presented (Potter, Am J Hum Genet, June 1991) that explains both the familial and sporadic forms of Alzheimer's disease as arising from the overexpression of or dysregulation of chromosome 21 in the life of the individual. That is, trisomy 21 cells, developing over time by unequal chromosome segregation during meiosis, may ultimately lead to cases of Alzheimer's disease through the same (as yet unknown, and perhaps multistep) mechanism by which Down syndrome patients get the disease, but at a later age due to the modulating effect of the mosaicism. The propensity to develop trisomy 21 cells could be genetic in origin (either due to an aberrant chromosome 21 centromere or to a mutation occurring elsewhere in the genome and affecting all chromosomes segregating), or it could be caused by environmental factors. In situ hybridization studies are being carried out to determine the extent of trisomy 21 mosaicism in the skin and cerebral nervous system of familial and sporadic Alzheimer's disease cases.

423.19 EFFECT OF AGING ON NA,K-ATPASE ACTIVITY IN HUMAN FRONTAL CORTEX. B.A. COLVIN, L. WINEC, A. WU, and N. DAVIS. Zoological and Neurosciences Institute, Ohio University College of Osteopathic Medicine, Athens, OH 45701. Cerebral plasma membrane vesicles (PMV) were purified by sucrose density gradient centrifugation from either rat brain or frozen human postmortem frontal cortex tissue slices. Human specimens were obtained from the National Neurological Research Bank, Los Angeles, CA. An animal model was studied to test the effect of short term post-mortem delay on Na,K-ATPase activity. Rats were decapitated and their skulls were incubated at 0-4°C for various times up to 4 hrs. The brains were then removed and processed into PMV. No significant effect of post-mortem delay was seen on the purification, as judged by the activity of plasma membrane marker proteases. Micro-fragment of membrane-associated proteins, as judged by SDS-PAGE, was not evident even after 4 hr of incubation. No significant effect of post-mortem delay was seen on estimates of Na,K-ATPase exchange \( V_{\text{max}} \) or \( K_{\text{m}} \), or passive permeability. PMV from human frontal cortex samples (ranging in age from 20 to 90 yrs.) were assayed for Na,K-ATPase exchange \( V_{\text{max}} \) and \( K_{\text{m}} \), and passive Ca2+ permeability. At present, no age related effects have been observed in these parameters of Na,K-ATPase transportant in human tissue. Mean values obtained thus far are: \( V_{\text{max}} = 2.13 \pm 0.86 \text{nMol/mg/sec} \) and \( K_{\text{m}} = 1.43 \pm 0.06 \text{mM} \). The rate constant of Ca2+ release was \( 0.03 \pm 0.01 \text{min}^{-1} \). These results indicate that central plasma membrane Na,K-ATPase countertransport is extremely stable with respect to post-mortem delay. Although more human tissues must be assayed before final conclusions are made, preliminary studies indicate few, if any age related effects on plasma membrane Na,K-ATPase countertransport.

423.20 PRESENCE OF ABERRANT PROTEINS IN DORSAL ROOT GANGLIA PRODUCES INTRANEURAL INCLUSIONS CONTAINING CYTOSKELETAL COMPONENTS AND UBIQUITIN. P.L. Di Patre, N. Wicker*, Y. Chen*, L. Antilio-Gamberti, and P. Gambetti. Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, OH, 44106, USA. Formation of intraneuronal inclusions containing both normal and altered cytoskeletal proteins as well as ubiquitin is a feature of age-related neurodegenerative diseases. We hypothesized that expression of aberrant proteins in neurons of the dorsal root ganglia (DRG) of young rats might provide a model for the presence of aberrant proteins. To test this hypothesis, we studied the effect of canavanine (Can), an arginine analogue, on cultured rat dorsal root ganglia (DRG's). We previously characterized this model in which Can replaced Arginine, most neurons contained rounded cytoplasmic inclusions that were silver positive and immunoreacted with antibodies to phosphorylated and non-phosphorylated neuronal tubulin and ubiquitin. These findings indicate that a condition resulting in the presence of a large amount of aberrant proteins is associated with the formation of intracellular inclusions. The antigenic characteristics of these intraneuronal inclusions are similar to those of the inclusions present in neurodegenerative diseases. Thus, we suggest that the presence of aberrant proteins secondary to the disease process might play a role in the formation of neuronal inclusions of neurodegenerative diseases such as the neurofibrillary tangles of Alzheimer disease. Supported by NS 14509, AG-08012 and AGNS08155.

423.21 NEUROCHEMISTRY OF THE AMYGDALA IN ALZHEIMER'S DISEASE (AD). L.C. Moore. Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461. The importance of the amygdala in memory function has been established by lesion studies in animals. In man, bilateral lesions of the amygdala cause amnesia. Histological investigation of the amygdala in Alzheimer's disease (AD) has revealed the presence of senile plaques (SP) and neurofibrillary tangles (NFT), as well as reduced levels of volume nuclei of the amygdala. Absent SP are also found in the amygdala of non-demented individuals with AD, and hyperammonemia compared to non-hyperammonemic non-deranged (non-AD) controls, often at levels diagnostic of AD, while NFT are rarely found in the amygdala of these individuals. Using established methods we have investigated the mRNA expression of choline acetyltransferase (ChAT), serotonin and noradrenergic neurotransmission in whole amygdala from 19 age-matched individuals (5 AD, 3 hypertensive, 5 cCAD and 6 non-HD). Scatchard analysis for RM CP I. Restriction enzyme mapping indicates the presence of at least one other CP I species in this amplified band, corresponding to a second mammal CLIP. We have also used this approach to sub-clone and partially sequence an amplification product coding for CLIP I. Restriction enzyme mapping indicates the presence of at least one other CP I species in this amplified band, corresponding to a second mammal CLIP.

423.22 DIFFERENTIAL CELLULAR EXPRESSION OF MAO-A AND MAO-B IN CONTROL VS. ALZHEIMER BRAINS. J. Squart*, L. Shin, M. DiPaolo*, V. Chen, Pelayo*, U. Ulrich*, G. Hupert, L. Kommer and J.G. Richards. Pharma Division, Preclinical Research, Hoffmann-La Roche Ltd, CH-4002 Basel, 1 John Stuffer Pharmaceutical Sc. Center, USC, Los Angeles CA, Neurology Clinic, Kantonsspital, Zurich, and Pathology Institute, Kantonsspital, Basel, Switzerland. Monoamine oxidases (MAO) oxidatively deaminate neurotransmitter and serotonin-like amines in the CNS and periphery. Two forms (MAO-A and MAO-B) have been identified by substrate selectivity, inhibitor sensitivity and primary structure. Quantitative enzyme radiolucito-auto-grammy and in situ hybridization have now revealed the distribution, abundance and cellular localization of the isoenzymes and their mRNAs in human postmortem brain tissue. Whereas MAO-A, (protein) and mRNA expression is mediated by noradrenergic neurons of the locus coeruleus, MAO-B is expressed by serotonergic neurons of the raphe nuclei. In the substantia nigra, MAO-A is restricted to the zona compacta whereas MAO-B was found mainly in the zona reticulata. To date, mRNA for neither MAO nor MAO-B could be detected in the substantia nigra or in glial cells of this brainstem. In cerebral cortex and hippocampus but not cerebellum from Alzheimer individuals, MAO-B was markedly increased in astrocytes surrounding senile plaques (identified by α-amyloid and GFA antigens) and in situ hybridization of MAO mRNA could not be detected in plaques. The functional implications of the differential compartmentalization and regulation of these isoenzymes will be discussed.

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The efficacy of tetraydroaminodrine (THA) plus lecitin in the treatment of cognitive impairment in patients with probable Alzheimer's disease (AD) was compared to that of placebo. Eighty-nine patients entered the study; 24 were withdrawn due to side effects and 5 for other reasons. Patients received the maximum tolerated dose of up to 150 mg per day of THA and 10 mg of lecitin. They were randomly assigned to double-blind treatment of either active or placebo drugs crossing over after 13 weeks treatment and 4 weeks washout. An analysis of covariance was performed on the trial showed a statistically significant beneficial effect of THA plus placebo on the Mini-Mental State Examination with 45% showing an improvement of 3 or more points compared to 11% in the placebo phase. Similar changes occurred in the Abbreviated Mental Test Score. There was however a considerable inter-subject variability in response. It is concluded that THA produced a demonstrable improvement in key outcome measures robustly equivalent to the deterioration which might have occurred over 6 - 12 months. Significant improvements were also seen on tests of attention but not memory as measured by the CANTAB batteries. Results are discussed in terms of the role of the cholinergic system in the control of attention and information processing.


Alzheimer's disease (AD) is characterized by marked loss of neuronal and cholinergic innervation in the neocortical regions of the brain. A decrease in frontal choline acetyltransferase (CHAT) activity has been correlated with slowing of the EEG and widening of the third ventricle. To test this correlation in AD, we performed a 20 subject autopsy series. 10 patients with the dominant neurofibrillary tangle pathology had CHAT activity for patients with the dominant occipital rhythm of 5-6 Hz (n=7), 7 Hz (n=8) and 8-9 Hz (n=5) was 36 ±7, 61 ±16 (mean±SEM, pmol/mg protein/min), respectively, the control values being 94 ±16.5; the groups with higher and slower EEG slowed significantly (p=0.01). The width of the third ventricle correlated with the frontal CHAT activity (r=0.65, p<0.05). The finding supports the contribution of the cholinergic deficit to EEG slowing in AD. The width of the third ventricle may partly reflect cholinergic changes in the basal forebrain and thus be related to the neocortical CHAT depletion.

423.27 ADENOSINE (A1) AND NMDA RECEPTORS IN THE HIPPOCAMPUS AND PARAHIPPOCAMPAL GYRUS OF ALZHEIMER'S DISEASE BRAINS J. Reynolds Tobacco Co. Orange, CA 92867, USA.

Adenosine acting via presynaptically located A1 receptors can inhibit the release of several neurotransmitters including the potentially neurotoxic excitatory amino acid glutamate, implicated in the pathology of Alzheimer's disease (AD). Our previous studies have demonstrated a significant decrease of binding to the NMDA receptor in AD brains. A specific A1-associated site in the CA1 hippocampal region of AD brains can be blocked by specific A1 antagonists. In the present study, we investigated the association of A1 receptors with NMDA receptors in the temporal, frontal and parietal regions of AD brains using autoradiographic techniques. A1 receptor binding was performed on sections of AD brains, with a specific A1 antagonist. The results show that A1 receptors are significantly decreased in AD brains, while NMDA receptor binding is preserved. The decrease in A1 receptor binding is significantly correlated with the severity of the disease. These findings suggest that A1 receptors may play a role in the pathophysiology of Alzheimer's disease.


Collection of human brains must be preceded by the analysis of fixation and storage conditions. We searched for the optimal preservation of both acetylcholinesterase and butyrylcholinesterase, which accumulate in senile plaques and neurofibrillary tangles. Three fixatives were evaluated: (A and B) 4% paraformaldehyde + 0.1% glutaraldehyde and (C) 4% formaldehyde + 0.1% glutaraldehyde + 0.1% picric acid. For longer periods (a year or more), tissue was stored at 4°C in 0.1 M phosphate buffer. The best fixative for both enzymes was A or C, with no significant difference in the optimal fixation-times (48 hr). With B both enzymes were reduced after short fixation-times (24-48 hr), and completely inactivated after longer fixation-times (5-6 days). The best fixative for both cholinesterases was A, and the optimum fixation-time was 48 hours. With B both enzymes were reduced after short fixation-times (24-48 hr), and completely inactivated after longer fixation-times (5-6 days). In general, the use of formaldehyde collections with C was less effective than with B. Glutaraldehyde can be used for a few hours to improve the EM. ultrastructure. Fixed blocks were stored at 4°C in 0.1 M phosphate buffer plus 10% sucrose for 3-3 months before a reduction of butyrylcholinesterase was observed. For longer periods (a year or more) blocks were kept frozen at -70°C in a 30% gelatin + 30% ethyl buffered solution, without further decrease of enzymatic activity. Tissue already sectioned was successfully stored frozen at -170°C for up to 4 months in the glycol solution. Supported by FIS 88/0922 and 90/0259.

A loss of dopamine (DA) containing neurons appears to be the primary pathogenetic changes in Parkinson's disease. The cause of DA cell death remains unclear. Since paraquat is chemically similar to MPTP and was implicated in causing Parkinsonism's symptom in humans, we have studied the action of paraquat on catecholamine containing cell lines. We have demonstrated that the rat pheochromocytoma cell line (PC12) can actively take up both dopamine and paraquat. Incubation of cells with paraquat leads to some cell death in a dose- and time-dependent manner. We have also demonstrated that pretreatment of cells with paraquat at various time intervals have led to protection in dopamine uptake activity. Since oxygen radicals may be involved in the toxicity of paraquat as demonstrated (Yang et al., Free Radical Biol. Med. 9 Suppl. 1, 165, 1990). Our results correlated well with synaptosomal DA uptake and lipid peroxidation activity after mice treated with paraquat. It appears that the neurotoxins, such as MPTP and paraquat may cause Parkinson's disease by free radical attack on dopaminergic neurons.

The possible protective effect by free radical scavengers and pheochromocytoma cell line (PC12) can actively take up both primary pathological changes in Parkinson's disease. The cause of DA using the cell model.


The antiparkinsonian effects of dopamine agonists have largely been attributed to accretion of D2 dopamine receptors. Reports in human and non-human primates using SKF83986, the prototypic D1 agonist, have failed to demonstrate significant antiparkinsonian effects, suggesting that D1 receptors were not involved. Based on stimulation of CAMP synthesis, SKF83986 is only a partial agonist. Recently, dihydrixidine has been reported to be a full D1 agonist, bioavailable in brain, and to have a ten fold D1: D2 selectivity. We have therefore administered dihydrixidine to MPTP-treated non-human primates. MPTP produces a parkinsonian syndrome in humans and in non-human primates. In Parkinson's disease and the MPTP-treated primate, motor defects are correlated with regional dopamine losses and decreased eye blink rates. Dihydrixidine (0.3-0.9 mg/kg, i.m.) significantly reversed parkinsonism and increased eye blink rates in five parkinssonian MPTP-treated African green monkeys in a "blind", pseudorandom sequence, saline-controlled study. Behavioral measures of parkinsonism were markedly reduced for 30-45 minutes after the drug, while eye blink rates and healthy behaviors increased. After 45 minutes, the monkeys returned to their prior level of disability. Antiparkinsonian effects were correlated with changes in blink rate. Our pharmacological studies show that blink rate can be independently regulated by D1 and D2 receptors. Antiparkinsonian effects indicate that it and other full efficacy D1 receptor agonists may be of particular utility in the treatment of Parkinson's disease.

Support by NS34075, ES01004, RSA M300643 to DER. Burroughs Wellcome fund to RMB.

MPTP (3,4-methylenedioxyphenyl-2,5-tetrahydroisoquinoline) is neurotoxic to neurons containing dopamine in the substantia nigra. It has been used to produce experimental Parkinson's disease. Apomorphine has been used to treat L-Dopa induced dyskinesia. However, it has not been tested in MPTP monkeys.

MPTP was microinjected into the ventral striatum in 4 monkeys. Apomorphine was administrated intravenously by a pump. It was left on for 2 months. No side effects were observed. The MPTP IV infusion rate was 0.5 mg/kg/hr.

This study suggests that apomorphine can be used to treat L-Dopa induced dyskinesia in MPTP monkeys.
424.13 STRIATAL TRANSPLANTATION OF BOVINE CHROMAFFIN CELL-Loaded MICROCAPSULES REDUCES EXPERIMENTAL PARKINSONISM IN RATS S.R. Winn1, B.A.E. Bakay2 and A. Mandi3. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322. Striatal transplantation of catecholamine-releasing cells encapsulated within a self-expanding membrane may provide a strategy for dopamine replacement in animal models of Parkinson's and prevent immune rejection in xenotransplants. Immobilizing adult bovine adrenal chromaffin cells in a polyelectrolyte matrix prevents fusion of cellular aggregates which may develop necrotic cores due to inadequate nutrient support. Coating an NGF-releasing core cell line was attempted to transform BAC cells from an endocrine to a more neuronal phenotype. In vitro, cell-microcapsules were characterized by observing basal, amphetamine and high K+ evoked release of catecholamines and observing cell viability over time. Cells within the microcapsules continued to survive for at least 12 weeks in vitro. Short neurites were seen extending from the coated BAC cell lines after 4 weeks in vitro. This differentiation increased amphetamine and high K+ stimulated dopamine output from 1 to 4 weeks that was maintained for up to 12 weeks in vitro. BAC-loaded microcapsules were implanted into 6-OHDA unilaterally lesioned striata of rats. From one to four weeks post-implantation, BAC cell-loaded recipients (n=6) showed a 50% reduction in rotation behavior under amphetamine challenge as compared to animals which received empty microcapsules (n=6). In vivo, microcapsules containing DBH and TH immunopositive BAC cells were observed after 4 weeks of implantation in animals exhibiting a reduction in turning behavior. Immunization of discrete cell clusters in polyelectrolytes prevents reaggregation and may provide a method for enhancing long-term maintenance of chromaffin cells.

424.14 QUANTIFYING BEHAVIORAL IMPROVEMENT IN HEMI-PARKINSONIAN MONKEYS FOLLOWING CNS TRANSPLANTATION OF BOVINE CHROMAFFIN CELL-Loaded MICROCAPSULES. R.A.E. Bakay1, B.A.E. Bakay2, and A. Mandi3. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322. One of the most difficult problems in evaluating the success of CNS transplantation techniques is obtaining a quantitative measure of the disability produced by the parkinsonian model. Our experience with MPTP hemiparkinsonian monkeys suggest that behavioral not a satisfactory index of disability. In an attempt to obtain a more representative quantitative analysis, monkeys were trained to perform a discrete-trial operant task which required the simultaneous depression of two levers on a response panel. Once both levers were depressed, lights above the right or left lever were darkened to indicate which hand to use in touching an illuminated area on a touch-sensitive screen. Correct responses were rewarded with a food pellet. Highly stable and accurate performance with both hands was established prior to MPTP injection. Testing allowed the separation of hand functions into response time, movement time, precision and accuracy of the responses, and provided a quantitative behavioral measure of parkinsonism. Preliminary results with this technique suggest quantitative improvement after transplantation procedures which yielded viable grafts as opposed to those performed on a sham basis. The smaller the MPTP-induced deficit, the more likely the behavioral improvement. Supported by VARAD, RO1 NS24340 and RR-00165.

424.15 EXPRESSION OF TYROSINE HYDROXYLASE IN STRIATAL CELLS WITH A HSV-1 VECTOR CAUSES STABLE PRODUCTION AND REGULATED RELEASE OF DOPA AND DOPAMINE: POTENTIAL USE FOR PARKINSON'S DISEASE. A. Froges1, M.J. Durr1, D.K. O'Malley1, and A. Geller2. Dept. of Neurosurg., Hosp. Univ. of PA., Phila. PA. 19104; Dept. Neurosurg., Yale Univ. Sch. of Med., New Haven CT. 06510; Dept. Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis MO. 63110; Div. of Cell Growth and Regulation, Dana Farber Can. Inst., Boston MA. 02115. In recent years, retroviral vectors have been used to causally transfect mammalian neural cells. HSV-1 vectors are typically used to deliver proteins into neurons or neural cells of nerve tissue. We constructed a retrovirus vector (pHSVth) which expresses the human TH gene from the HSV-1 IE 4.5 promoter, a constitutive promoter active in most neural cells. Fibroblasts inoculated with pHSVth contain TH DNA, TH RNA, and TH immunoreactivity. TH immunoreactivity is detected in approximately 10% of the cells. Furthermore, both L-dopa and dopamine were increased in both L-dopa and dopamine levels. Cultured striatal cells were infected with pHSVth, one week later TH immunoreactivity was detected in approximately 10% of the cells. pHSVth infected fibroblasts also contain a 90-fold increase in TH enzyme activity and a 10-fold reduction was observed following the implantation of empty polymer capsules which was comparable to extracellular DA levels of unlesioned controls. To further clarify if the observed rotation in reduction in rotation was mediated by chronic stimulation of DA receptors secondary to its diffuse release from the PC12 cell-containing capsules, a fluoscence PC12 cell-containing encapsulation system was implanted into the 6-OHDA lesioned striata of rats. Only cell-containing devices reduced the apomorphine-induced rotational response. Moreover, one week after removing the PC12 cells by flushing the U-shaped encapsulation system the effect was reversed. Viable cells were confirmed by staining with a fluorescent vital dye. The reversibility suggests that release of a neurotransmitter, most likely DA, was responsible for the observed functional effect using this cell line.

424.16 QUANTIFYING IMPROVEMENT FOLLOWING CNS TRANSPLANTATION IN HEMI-PARKINSONIAN MONKEYS USING AN OPERANT BEHAVIOR TASK. R.A.E. Bakay1, B.A.E. Bakay2 and A. Mandi3. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322. One of the most difficult problems in evaluating the success of CNS transplantation techniques is obtaining a quantitative measure of the disability produced by the parkinsonian model. Our experience with MPTP hemiparkinsonian monkeys suggests that behavioral not a satisfactory index of disability. In an attempt to obtain a more representative quantitative analysis, monkeys were trained to perform a discrete-trial operant task which required the simultaneous depression of two levers on a response panel. Once both levers were depressed, lights above the right or left lever were darkened to indicate which hand to use in touching an illuminated area on a touch-sensitive screen. Correct responses were rewarded with a food pellet. Highly stable and accurate performance with both hands was established prior to MPTP injection. Testing allowed the separation of hand functions into response time, movement time, precision and accuracy of the responses, and provided a quantitative behavioral measure of parkinsonism. Preliminary results with this technique suggest quantitative improvement after transplantation procedures which yielded viable grafts as opposed to those performed on a sham basis. The smaller the MPTP-induced deficit, the more likely the behavioral improvement. Supported by VARAD, RO1 NS24340 and RR-00165.
425.1

Several lines of evidence now support the concept that lactacidosis plays a key role in determining the outcome of brain anoxia/ischemia. Our previous studies on brain of rats and mice and the I and a day old neonatal rat demonstrated that lactate induces the brain cytosol acid buffering ability and plays a fundamental role in the immature brain's high resistance against anoxia. In this study, we tested the protective effects of guardinodithane sulfate (GES), a lactate analogue, on brain lactate generation in adult mice. The data indicated that GES can increase the brain acid buffering ability and effectively prevents severe acidosis from developing during anoxia. GES greatly enhanced the survival rate of mice exposed to anoxia.

<table>
<thead>
<tr>
<th>Survival rate</th>
<th>Duration of Anoxia</th>
<th>GES</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 minutes</td>
<td>50-60%</td>
<td>50-60%</td>
</tr>
<tr>
<td></td>
<td>2.75 minutes</td>
<td>80-90%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Each experiment consisted of 5 mice and 5 control animals and experiment was repeated three times.

425.3

We reported last year that Honghua, an extract of safflower used as an herbal medicine in China, has neuroprotective activity in experimental models of excitotoxicity. In the in vivo adult rat retina, ischemic damage induced by dye photocoagulation was greatly reduced by intravitreal injection of Honghua. In the isolated chick embryo retina (CER), Honghua protected against the excitotoxicity of glutamate receptor agonists, N-methyl-D-aspartate, kainic acid and quisqualic acid, and against neuronal degeneration caused by simulated ischemia (30 min glucose/oxygen deprivation). Subsequently, we found that Honghua more potently protected isolated simulated ischemia-induced retinal neuronal degeneration against the agonists. Biochemical fractionation procedures were used to purify an active component of Honghua. An approximately 100-fold purification of an active principle was achieved using ion-exchange, gel filtration, and reverse-phase chromatography. The purest fractions were determined to be rich in glucose, so the effects of glucose in the models of excitotoxicity were determined. Many of the neuroprotective effects of crude and highly purified Honghua were mimicked by pure solutions of equivalent glucose concentration. Concentrations of glucose ≥1.2 mM in the CER-ischemia assay provided complete protection from neurotoxicity, despite the absence of oxygen. Intravitreal injection of 1.5 μmol of glucose provided highly significant protection (67% reduction in retinal damage determined histologically at 1 hr post ischemia; n=14, p<0.001) in the adult rat retina dye photocoagulation model. It should be noted that substances injected intravitreally diffuse freely into the ischemic retina. Thus, direct delivery of glucose to ischemic neurons in the immediate period following CNS vascular occlusion (stroke), delays progression of neuronal degeneration for at least 1 hr. These findings have relevance for the management of retinal vascular occlusion and possibly other neuro- ischemic disorders. This work was supported by grants from Fight-for-Sight (CR) and NIH (JOW).

425.5

Recently, BMY-14802, a putative sigma antagonist and antipsychotic agent, and Clozapine, an atypical antipsychotic agent, were shown to synergize in the treatment of ischemia and depolarized ischemia induced increases in cGMP in the cerebellum, a well characterized second messenger response mediated by the NMDA receptor-channel complex. The purpose of this study was to assess whether BMY-14802, Clozapine, and other NMDA antagonists, also be neuroprotective in an in vivo model of ischemia. Using the gerbil model of ischemia, BMY-14802 was found to be significantly protective against ischemia. BMY-14802 and Clozapine when co-administered were synergistic in protecting the animals from ischemia. These results suggest that BMY-14802 and Clozapine may be useful in attenuating ischemia-induced injury in the CNS.

425.2
DIETARY NONPROTEIN CALORIES AND CEREBRAL INFARCTION SIZE. C.B. Robertson*, J.C. Goodman, R.G. Grossman, Department of Neurosurgery, Baylor College of Medicine, Houston, TX 77030.

The effect of reducing carbohydrates in the diet on cerebral infarction size was studied after reversible middle cerebral artery occlusion (MCAO). Rats were either fasted for 24 hours prior to MCAO or were fed a control diet containing 51.5% of calories as carbohydrates, or one of 5 experimental diets in which 60% of the carbohydrate calories were replaced with one or more of the following: fat, sucrose, starch, fructose, triacetin, and tributyrin. The lowest plasma glucose concentration was achieved in the fasted group, 6.5±1.1 mmol/ml, and the highest was found in the 1,3-butanediol group, 9.2±1.4 mmol/ml. The 1,3-butanediol diet resulted in a plasma glucose concentration of 7.8±1.3 mmol/ml. The smallest infarct volume was found in the fasted group, 53±43 mm3, and the largest in the control diet group, 162±56 mm3. Significantly smaller infarcts were found with the 1,3-butanediol diet, 98±41 mm3, and with the triacetin/tributyrin diet, 105.4±52.6 mm3. The size of the infarction was directly related to the preischemia blood glucose concentration (r=0.79, p<0.05). It may be possible to develop a diet using nonglycolytic calorie sources which would supply systemic caloric and protein requirements without the adverse effect of conventional diets.

425.4

Asphyxia to male Sprague-Dawley rat pups was induced by a delayed cesarean section (Bjelke et al., 1991). Immunohistochemical and functional analyses were carried out on the pups at an age of 3 weeks. It was demonstrated that asphyxia time-dependently produced an increase in the number of tyrosine hydroxylase immunoreactive (TH-IR) nerve cell bodies in the substantia nigra. Furthermore, asphyxia time-dependently reduced rearing behavior and increased locomotion (Bjelke et al., 1991). In the present study the pups received nicotine via the breast milk of dams implanted with an Alzet minipump (0.125 mg nicotine/kg/h for 3 weeks). It was found that nicotine treatment counteracted the asphyxia-induced increase in the number of TH-IR nerve cell bodies of the substantia nigra as well as the changes in rearing and locomotor behavior.

In conclusion, nicotine treatment of male rat pups can counteract asphyxia-induced changes in nerve cell body number in the substantia nigra, as well as changes in locomotor behavior.


425.6

Fosphonyl is a water soluble prodrug of phentoyin that is rapidly converted to phentoyin by non-specific phosphatases in plasma. Previous experiments with phentoyin have indicated that it has neuroprotective effects in models of both global and focal ischemia. These experiments were conducted to determine if fosphonyl would decrease the size of ischemic damage in a distal model of middle cerebral artery (MCA) occlusion and decrease the neurological deficits caused by a proximal MCA occlusion. In halothane anesthetized Fisher-344 rats the common carotid and ipsilateral MCA were occluded. Drug or vehicle was administered IV, 30 minutes and 24 hours post occlusion and the infarct was measured using TTC staining 48 hrs post surgery. In the distal MCA occlusion model fosphonyl was tested at 17.3, 30, 60 and 100 mg/kg (n=12 per group) and significantly decreased the infarct volume at each dose by 22%, 35%, 39% and 26% respectively as compared to vehicle treated rats. In the proximal MCA occlusion model fosphonyl was tested at 30 mg/kg and decreased the infarct volume by 57%. These animals were tested for neurological deficits by measuring the duration they remained on a 50°C inclined plane. Fosphonyl reduced neurological deficits to shams levels as compared to vehicle treated rats. These experiments suggest that fosphonyl reduces ischemic damage and may be useful in the treatment of human stroke.
425.7 CYCLOHEXIMEDE OFFERS PROTECTION AGAINST THE DELETIONAL INFLUENCES OF ANOXIA IN THE RAT. S. Papas, D. Arendash*, W. Yang, P. Chiba, and Y. Ben-Ari. INSERM Unit 29, 123 Bd. de Port-Royal, Paris, 75014, France

Forebrain ischemia in the rat results in decreased protein synthesis and delayed neuroprotective effects. However, treatment of these rats with cycloheximide (CHX), a protein synthesis blocker, prevents DND in CA1 (Goto et al., 1990), implicating protein synthesis as a mediator of neuroprotection. We have recently observed in vivo and in vitro effects of CHX on the influences of anoxia in the rat.

Adult male Wistar rats were subjected to forebrain ischemia (4- vessel occlusion) for 5 min and monitored for 7 days. Rats were injected i.p. with saline or CHX (1.5 mg/kg) 1 h before or after occlusion. CHX treatment prior to ischemia decreased (+p<0.05, student’s t-test) weight loss (2.5±1.0 g vs. 0.9±0.8 g, mean±SEM) and enhanced survival rate (60% vs. 48% in controls) of rats. Weight loss was similar to controls (+p<0.05) and survival rate (25%) decreased in rats CHX after occlusion. Hence, protein synthesis occurring during or within 1 h of insult may mediate in vivo effects of forebrain ischemia.

Effects of anoxia on CA1 field EPSPs in the presence of CHX were also demonstrated. EPSPs in adult hippocampal slices incubated in Krebs (controls) or 60 μM CHX were subjected to various periods (3 min 15 s to 4 min 30 s) of anoxia (glucose-free Krebs, 95%N2/5%CO2 perfusion) and their percent recuperation determined 20 min later. EPSP recuperation in control and CHX slices was similar for all ranges of anoxia, except 3 min 45 s. After this period, CHX treated slices exhibited a greater (+p<0.05) recuperation (57.5±17.0%) than controls (11.9±11.0%). CHX may thus enhance CA1 resistance to anoxia, and some of its protective effects may occur in the hippocampus.

425.9 DIAZEPAM FOLLOWING CEREBRAL ISCHEMIA PRESERVES CA1 PYRAMIDAL CELLS AND GABA RECEPTORS OF THE HIPPOCAMPUS. Robin A. Huff and Formelle D. Schwartz, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

Cerebral ischemia-induced neuronal injury in selectively vulnerable areas is associated with excessive excitatory neurotransmission. We investigated whether enhancement of inhibitory neurotransmission could protect hippocampal neurons from ischemic damage in gerbils subjected to bilateral carotid occlusion (BCO) for 5 min. Diazepam (DZ), a drug that enhances GABA neurotransmission, was injected into sham-operated and occluded gerbils during the reperfusion phase. Post-ischemic hyperactivity was measured as a percentage of CA1 hippocampal damage 1 day following the occlusion. Seven days after the occlusion, CA1 damage was assessed by histologic procedures, and the distribution of the GABA receptor-gated chloride current was assessed by [3H]GABA autoradiography. One day following BCO, gerbils showed a robust increase in locomotor activity, whereas controls were not affected. Diazepam dose-dependently decreased CA1 damage 1 day following ischemic injury. Diazepam also decreased [3H]GABA binding in the CA1 stratum oriens. Post-ischemic hyperactivity was significantly decreased following ischemia. Diazepam completely protected BCO gerbils from this loss of [3H]GABA binding. These results indicate that 1) DZ given after an ischemic insult is neuroprotective of CA1 pyramidal neurons, and 2) the neuroprotective effect of DZ prevents the loss of GABA receptors residing on CA1 pyramidal cell dendrites. We conclude that enhancement of GABA neurotransmission following an ischemic event may limit excitatory neurotransmission and thereby prevent neuronal death.


Treatment of experimental stroke with opiate agonists has provided variable data. Previous models of focal ischemia involved occlusion of only the middle cerebral artery (MCA). In the current study, 20 min of 3 vessel occlusion of 2mm MCA, anterior cerebral and intracranial internal carotid arteries. Six hours post occlusion half of the cats received an injection of 2mg/kg dynorphin (1-13) (DYN) and a saline (SAL). Cats were sacrificed on the eighth post-operative day and their brains stained with TTC.

Survival was 10% for SAL and 50% for DYN. Abnormally stained tissue was categorized into infarct (colorless) and ischemia (lightly stained). The data suggests that tissue that would have been infarcted without treatment, recovered following DYN treatment. Our data is consistent with the theory that kappa opioid receptor agonists have a therapeutic anti-edema effect in cerebral ischemia. The three vessel occlusion model shows promise as a model for experimental stroke.

425.12 ANOXIC DEPRESSION OF NEOCORTICAL SYNAPTIC POTENTIALS. A. S. Rosen and M. E. Morr, Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

During the depolarization (AD) which brief anoxia (5 min) evoked in pyramidal neurons of layers III-IV of rat neocortical slices (Rosen & Morris 1991, Neurosci. Lett. 124:169) both EPSPs and IPSPs are depressed. The early (e) EPSP is decreased by 50%-75% at 6 min while the late (l) EPSP is abolished within the first 2 min; persistence of these changes during temporary V. restoration indicates a mecha­nism distinct from their post-synaptic occlusion by AD. EPSP amplitude fully recovers at 6-8 min of reoxygenation. Evoked IPSPs disappear even sooner than the l EPSP and their recovery is more prolonged.

When temperature is changed from a control level of 33.5°C to 30° or 37° warming depolarizes the membrane slightly and reduces the amplitude of both EPSPs and IPSPs causing reductions and delays anoxic-evoked depression of post-synaptic potentials and hastens recovery; warming has opposite effects. The peak amplitude of e EPSP is significa­ntly altered, although the rising phase is slower at 30°. These findings show that in the neocortex anoxia produces a consistent depression of synaptic transmission, which can be opposed by hypothermia.

(Supported by the Medical Research Council of Canada.)

Histopathological studies indicate that mild brain hypothermia can attenuate the neuronal and microvascular injury following complete global ischemia in rodents (e.g., Busto et al., 1987). Although histopathological measures can provide a gnosiological status of an animal, they do not reveal the functional integrity of an affected brain region. The present study evaluated the role of hypothermia can influence behavioral outcome following ischemia induced by transient 4 vessel occlusion (4VO).

Postischemic cell change was graded blindly, on a 0-3 scale following 7-day survival. In area CA1 of hippocampus, Groups A and B showed significantly fewer CA1 neurons than the Sham (p<0.05) or the Group C (p<0.01) rats. In contrast, in the neostriatum and thalamus, there were no group differences in any of the semiorientor measures or in the performance on the simple place task. In the hippocampus, the survival of CA1 pyramidal cells correlated only with the difference between the preischemic baseline and the mean brain temperature for the first 30 min after ischemia. Maintaining intraischemic brain temperature at a nearly physiological value diminishes both the influence of postischemic brain temperature (BT), measured by a thermistor in frontal cortex, and BT was raised to 39°C by a warming lamp over the head. Striatal extracellular fluid was sampled by microdialysis 30 min prior to and during ischemia, and for 4 hr following recirculation. Dialysate levels of GABA, glycine and glutamate were measured by HPLC. During ischemia, there was a 41-fold rise in GABA at 37°C and a 56-fold rise at 39°C. Glutamate levels increased 3-fold in Group 1 and 5-fold in Group 2 within 30 min of ischemia and remained high during recirculation. Glutamate levels rose 21-fold in Group 1, but in Group 2 there was a 37-fold rise over baseline. The excitotoxic index, reflecting the net influence of excitatory and inhibitory neurotransmitters (glycine x glutamate/GABA), was elevated only 2-fold during recirculation in Group 1, but in Group 2 there was a 20-fold increase over baseline. We conclude that mild cerebral hypothermia improved the functional outcome of ischemic brain injury.

ELEVATED BRAIN TEMPERATURE MAY ADVERSELY INFLUENCE THE OUTCOME OF AN ISOELIC INJURY. Using microdialysis, we examined neurotransmitter release in rats subjected to 20 min of forebrain ischemia by 2-vessel occlusion plus hypothermia (45 mmHg). In Group 1 (n=5), intraischemic brain temperature (BT) was maintained at 37°C. In Group 2 rats (n=6), BT was raised to 39°C by a warming lamp over the head. Striatal extracellular fluid was sampled by microdialysis 30 min prior to and during ischemia, and for 4 hr following recirculation. Dialysate levels of GABA, glycine and glutamate were measured by HPLC. During ischemia, there was a 41-fold rise in GABA at 37°C and a 56-fold rise at 39°C. Glutamate levels increased 3-fold in Group 1 and 5-fold in Group 2 within 30 min of ischemia and remained high during recirculation. Glutamate levels rose 21-fold in Group 1, but in Group 2 there was a 37-fold rise over baseline. The excitotoxic index, reflecting the net influence of excitatory and inhibitory neurotransmitters (glycine x glutamate/GABA), was elevated only 2-fold during recirculation in Group 1, but in Group 2 there was a 20-fold increase over baseline. We conclude that mild cerebral hypothermia improved the functional outcome of ischemic brain injury.

ROLE OF HYPOThERMIA IN MULTIPLE CEREBRAL ISCHEMIC INSULTS. B. Lin, W.D. Dietrich, M.Y.-T. Globus, R. Busto, and M.D. Ginsberg. Cerebrovascular Disease Research Center, Univ. of Miami School of Medicine, Miami, FL 33101.

To evaluate the cerebroprotective effects of hypothermia for repeated ischemic insults, we subjected male Wistar rats to three 5-min periods of global forebrain ischemia (by bilateral carotid artery occlusions plus hypothermia to 30°C for 15±5°C) separated by 60 min of normothermic recirculation. Rectal temperature was held at 37.0-37.5°C. In Group A (n=5), brain temperature (BT) measured by a thermistor in frontal cortex, was thermostated at 36.5-37.0°C throughout. In Group B (n=6), BT was held at 36.5-37.0°C during ischemia but was reduced to 30°C for 30 min following the first insult, and for 60 min following the third insult. Ischemic brain cell change was graded blindly, on a 0-3 scale following 7-day survival. In area CA1 of hippocampus, Groups A and B showed significantly fewer CA1 neurons than the Sham (p<0.05) or the Group C (p<0.01) rats. In contrast, in the neostriatum and thalamus, there were no group differences in any of the semiorientor measures or in the performance on the simple place task. In the hippocampus, the survival of CA1 pyramidal cells correlated only with the difference between the preischemic baseline and the mean brain temperature for the first 30 min after ischemia. Maintaining intraischemic brain temperature at a nearly physiological value diminishes both the influence of postischemic brain temperature (BT), measured by a thermistor in frontal cortex, and BT was raised to 39°C by a warming lamp over the head. Striatal extracellular fluid was sampled by microdialysis 30 min prior to and during ischemia, and for 4 hr following recirculation. Dialysate levels of GABA, glycine and glutamate were measured by HPLC. During ischemia, there was a 41-fold rise in GABA at 37°C and a 56-fold rise at 39°C. Glutamate levels increased 3-fold in Group 1 and 5-fold in Group 2 within 30 min of ischemia and remained high during recirculation. Glutamate levels rose 21-fold in Group 1, but in Group 2 there was a 37-fold rise over baseline. The excitotoxic index, reflecting the net influence of excitatory and inhibitory neurotransmitters (glycine x glutamate/GABA), was elevated only 2-fold during recirculation in Group 1, but in Group 2 there was a 20-fold increase over baseline. We conclude that mild cerebral hypothermia improved the functional outcome of ischemic brain injury.
HYPERTHERMIA PROTECTS SOMATOSTATIN NEURONS IN RAT DENTATE HILUS FROM ZINC ACCUMULATION AND FAST ISCHEMIC CELL DEATH. F.F. Johansen, N. Tønder1, J. Zimmer1, N.H. Døiø2. Pharmacology, Institute of Neuropathology, University of Copenhagen, and 1Institute of Neurobiology, University of Aarhus, Denmark.

We have previously shown that somatostatin containing neurons in the rat dentate hilus are highly susceptible to ischemic brain damage. Within 48 hours after ischemia they demonstrate fast ischemic cell death. Simultaneously, we have shown that necrotic cells in dentate hilus accumulate free zinc in their cytoplasm. We have now demonstrated directly that somatostatin containing cells in dentate hilus also accumulate zinc during maturation and ischemic cell death. Since it is known that hypothermia ameliorates ischemic brain damage, we studied whether hypothermia (29°C) protected the highly susceptible somatostatin containing neurons in dentate hilus from ischemic cell death and zinc accumulation. It was demonstrated that hypothermia during cerebral ischemia prevented necrosis and neuronal zinc accumulation in dentate hilus. Hypothermia possibly prevents excitotoxic glutamate release and associated translocation of zinc from the glial glutamatergic mossy fiber terminals to vulnerable somatostatin containing cells during ischemia. We find it less likely that zinc is released from intracellular binding sites during ischemic cell death, because necrotic located outside the mossy fiber layer do not show zinc accumulation during necrosis.


We have reported a cognitive deficit in a spatial learning task as the result of carotid occlusion (Barnes et al. 1990). Here we assess the effects of an ischemic insult on brain biochemical and histological parameters. Lister-hooded rats underwent 45 min bilateral common carotid artery occlusion under ketamine anesthesia (100mg/kg), and were either tested in a swim maze task or sacrificed for choline acetyltransferase (ChAT) analysis (Fonnum, 1975) or haematoxylin and eosin (H & E) staining.

Carotid occlusion resulted in a loss in learning ability (escape latencies for shams and occluded 16.52±1.97 and 110.54±5.52, p<0.001, one way ANOVA). ChAT activity (sham and occluded) was different as well in striatum (779.0±64.8 to 465.5±50.5 and 504.9±52.3 to 352.2±20.0 nmol/min/mg protein, P<0.05, t-test) whilst H&E staining showed no change in density of hippocampal neurons.

Therefore the loss of learning ability in the rat following an ischemic insult is not necessarily correlated with changes in ChAT activity or cell loss. Barnes & McRae (1990) Br J Pharmacol 101(Suppl), S69F Fonnum F (1975) J Neurochem 24, 407-409


Clinical studies have reported memory impairment and selective loss of hippocampal CA1 neurons after global ischemia. Significant memory impairment with selective CA1 cell damage have also been observed in the rat 4-VO model of global ischemia. The concordant pathophysiology of memory impairment and CA1 cell damage in man and in rat 4-VO model have suggested that the 4-VO model could be a useful model of "neuroprotective" efficacy of novel compounds after global ischemia. Remacemide, (2) 2-amino-N-(1-methyl-2-pyridyl)acetamide, is an anticonvulsant and NMDA antagonist currently undergoing human clinical trials. The effects of remacemide were evaluated functionally in terms of neuroprotective effects on memory of 4-VO memory-impaired rats in a T-maze, and morphotopically, by evaluation of cell density in hippocampus and CA1 regions. Remacemide, at 20 mg/kg, or saline was administered i.p. at 1 hour post-4-VO, and then once every 24 hours for 14 days. Brain sections were cut at 40μm and stained with cresyl violet. Paraformaldehyde. Brain sections were cut at 40μm and stained with cresyl violet.

Extensive neuronal loss was seen in the CA1 region in 2/6 hippocampi from cases which sustained 45 minutes of cardiac arrest at 20°C. Longer durations of cardiac arrest at lower temperatures (15-18°C) produced scattered patches of selective neuronal loss in the CA1 region in 2/6 hippocampi. All cases had evidence of partial loss of neurons from the regions of CA4 proximal to the granule cell layer. A partial loss of neurons from the medial portion of the fimbrial sector nucleus was also evident in some cases. These results suggest that hypothermic neuroprotection alone may not be sufficient to prevent the loss of neurons from selectively vulnerable structures during open heart surgery. Augmentation of the prophylactic administration of pharmacological agents may be necessary for ensuring complete neuroprotection during intra-operative ischemia.

426.1 IMPAIRED WATER MAZE ACQUISITION FOLLOWING CA1 ISCHEMIC DAMAGE IN THE GERBILL. D. Corbett, S. Nurse1, S. Evans2 and D. W. McKay. Fac. of Medicine, Memorial Univ. St. John's, NF, CANADA A1B 3V6.

The Morris water maze is a sensitive test of spatial learning that has been used to document deficits in rats that have extensive loss of CA1 neurons induced by forebrain ischemia (Auer et al., 1988). Similarly, we have found this test to be effective in gerbils subjected to carotid occlusion.

Gerbils were given 5 days (4 trials/day) of swim training (to a visible platform) in a circular pool filled with warm water. The next day, the gerbils were exposed to 5 min carotid occlusions under Halothane anesthesia (N=10) sham surgery (N=6) or left intact (N=6). Water maze acquisition took place 2 days later in a different testing room using a hidden platform and several conspicuous visual stimuli that were attached to the walls of the maze. Ischemic gerbils were slower to acquire the water maze task (F = 6.00, p < 0.05) than sham/normal gerbils although they eventually (7-10 days) learned to locate the platform. The Morris water maze has potential as a functional test in studies of cerebral ischemia in gerbils.

Supported by the MRC of Canada.


We have previously shown that both 15 and 30 min 4-vessel-occlusion ischaemia (4VO ISC) in the rat produce equivalent deficits in learning and memory for a spatial maze task and are accompanied by >90% hippocampal CA1 cell loss. The present study compared the equivalent deficits in learning and memory in a water maze (WM) and radial-maze (RM) performance. The first test (WM; 7 weeks post-op) confirmed that comparable spatial deficits are seen at 15 and 30 min ISC. In ISC rats latency to find the platform was increased relative to controls during acquisition (p<0.01), whilst time spent in the quadrant containing the platform was reduced (p<0.01). Both ISC groups showed an equivalent deficit in retention of the platform location in a probe trial. All rats were tested on place and cue tasks in the radial-maze (10-15 weeks post-op); no behavioural deficits were seen. Rats were retested on reversal learning in the WM (26 weeks post-op). ISC spatial deficits were similar to those seen in the first WM test. These data indicate that ISC can impair WM performance but spare RM performance. The requirement for more accurate use of allocentric spatial cues and the higher motivational demands of the WM may explain the difference between the two tasks. Since WM learning was still impaired after RM testing, recovery as a function of time or training cannot explain the lack of RM deficits. (Supported by the Wellcome Trust and the British Heart Foundation)
426.5

**4-VO INDUCED ISCHEMIA RESULTS IN CA1 CELL LOSS AND IMPAIRED 14-**


The four vessel occlusion (4-VO) model (Puttlinelli & Briery, Stroke, 10: 267, 1979) of transient global ischemia produces neuronal loss in hippocampus (HC), particularly CA1, and memory impairment in a variety of tasks. We used this model to assess the effect of CA1 damage in young rats on learning in a 14-unit T-maze that has provided robust evidence of age-related memory impairment (Ingram, Neurobiol. Aging, 9:475, 1998). Rats received either 15-min of 4VO ischemia, terminal artery blood flow (CBF) during middle cerebral artery occlusion (MCAo) in exposed neocortex of rats anesthetized with pentobarbital. K+o and CBF were monitored at multiple sites along the ischemic zone with suppressed electrical activity but preserved ion homeostasis. To better understand focal ischemia-induced pathophysiology of brain, present studies sought to spatially resolve extracellular potassium ion activity (K+o) and cerebral blood flow (CBF) during middle cerebral artery occlusion (MCAo) in exposed neocortex of rats anesthetized with pentobarbital. K+o and CBF were monitored at multiple sites along the cortical surface from the MCA clamp toward the midline of the brain by ion selective and polarographic (hydrogen clearance) microelectrodes implanted approx 500 μm within the cortical gray matter. At 1.3 mm medial from the MCA clamp, blood flow was reduced approx 75% and K+o was elevated to the 50-80 mM levels characteristic of anoxic depolarization (AD). This situation persisted for 3 hours after MCAo. Approx 7.8 mm medial from the clamp, blood flow and K+o were unchanged by MCAo during this period. Between these zones, intermediate CBF and K+o levels (the latter in the range of 20-35 mM) were recorded at 30 and 90 min following MCAo. At 180 minutes, however, these intermediate zones were less evident as K+o often increased in areas closer to the ischemic core to levels characteristic of AD while K+o sometimes decreased in loci adjacent to normal sites. The presence of intermediate K+o levels is unusual since regenerative spreading depression-like depolarization often accompanies elevation of K+o beyond approximately 10-15 mM. Identifying factors limiting K+o in intermediate zones during focal ischemia may provide new insights toward salvaging tissues after such insults.

426.6


Synaptophysin, a calcium binding protein, is present in presynaptic endings. Changes in the density of synaptic numbers and density are believed to be reflected in the amount of synaptophysin present. Using immunohistochemistry, The amount of synaptophysin is measured in the cortex in hypertensive and normotensive rats following ischemia. The insult is produced by permanent occlusion of the middle cerebral artery and the ipsilateral common carotid artery. Changes in synaptophysin are examined at time points of 3 days to 2 months following ischemia. We hypothesize that the differences in synaptophysin levels reflect the plasticity of the cortex following ischemia. This work is supported by NS 11255, NS 25400, RR 03779, NS 01217, and Bristol Myers-Squibb.

426.7

**SPATIAL RESOLUTION OF K+O AND CBF DERANGEMENT DURING MCA OCCLUSION IN RAT NEOCORTEX.** Z.C. Feng, T.J. Sirc and M. Rosenthal. Dept of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Focal cerebral ischemia may produce a penumbra area outside of the ischemic zone with suppressed electrical activity but preserved ion homeostasis. To better understand focal ischemia-induced pathophysiology of brain, present studies sought to spatially resolve extracellular potassium ion activity (K+o) and cerebral blood flow (CBF) during middle cerebral artery occlusion (MCAo) in exposed neocortex of rats anesthetized with pentobarbital. K+o and CBF were monitored at multiple sites along the cortical surface from the MCA clamp toward the midline of the brain by ion selective and polarographic (hydrogen clearance) microelectrodes implanted approx 500 μm within the cortical gray matter. At 1.3 mm medial from the MCA clamp, blood flow was reduced approx 75% and K+o was elevated to the 50-80 mM levels characteristic of anoxic depolarization (AD). This situation persisted for 3 hours after MCAo. Approx 7.8 mm medial from the clamp, blood flow and K+o were unchanged by MCAo during this period. Between these zones, intermediate CBF and K+o levels (the latter in the range of 20-35 mM) were recorded at 30 and 90 min following MCAo. At 180 minutes, however, these intermediate zones were less evident as K+o often increased in areas closer to the ischemic core to levels characteristic of AD while K+o sometimes decreased in loci adjacent to normal sites. The presence of intermediate K+o levels is unusual since regenerative spreading depression-like depolarization often accompanies elevation of K+o beyond approximately 10-15 mM. Identifying factors limiting K+o in intermediate zones during focal ischemia may provide new insights toward salvaging tissues after such insults.

426.8

**NEAR-TERM FOETAL RATS RESIST HYPOXIC NEURONAL INJURY.** L. Kendall*, S.P. Butcher* & J.S. Kelly. (SPON: Brain Research Institute, Department of Pharmacology, University of Edinburgh, U.K.)

Hypoxic and ischaemic insults during the perinatal period are known to be a major cause of non-progressive neurological deficits in humans. We have developed a model of perinatal hypoxia in anaesthetized rats which involves occlusion of the uteroplacental vessels of one uterine horn. After an occlusion period of 10, 20 or 30 minutes, which may or may not be followed by a reperfusion period, the pups are delivered by Caesarean section. The pups brains are extracted at 0, 1, 60 or 180 minutes for analysis of metabolites. The remaining pups are resuscitated then cross-fostered onto lactating dams and perfusion fixed at a later date for histological analysis. Following the most severe insult, a 30 minute occlusion, brain tissue levels of lactate are elevated from 6.1 ± 0.5 nmols/mg to 22.2 ± 1.5 nmols/mg tissue, ATP and phosphocreatine levels are reduced from 2.4 ± 0.5 nmols/mg to 0.9 ± 0.3 nmols/mg and from 1.0 ± 0.2 nmols/mg to 0.3 ± 0.2 nmols/mg tissue respectively. Histological investigations showed little or no damage 4 hours after the hypoxic insult however four to seven days post-injury discrete damage can be identified in some areas in the hind brain region.

426.9


To model accurately the clinical disorder of human thrombotic stroke, the present study used a photochemical reaction to induce focal thrombosis of the distal middle cerebral artery (dMCA) in two strains of rats. Male Wistar (n=9) and Sprague-Dawley(n=12) rats were anesthetized and prepared with arterial and venous catheters. The right dMCA was exposed above the frontal fissure. After i.v. injection of 3 mg grams of a photoactivating sensitizer, thrombosis was induced by irradiating the vessel with 20 mW from an argon laser activated dye laser tuned to 562 nm. The right common carotid artery (CCA) was then permanently occluded and the left CCA was temporarily occluded for 60 min. Animals were perfusion-fixed 3 days later and infarct volume was determined by measuring the volume of the right hemisphere. The right hemisphere of Sprague-Dawley rats had significantly larger cortical infarcts (x ± S.E. = 116.29 ± 17.35 mm3) than did Wistar rats (x ± S.E. = 47.88 ± 8.86 mm3). Blood pressure (BP) and Pco2 did not differ between groups either before or after an dMCA occlusion, during or after CCA occlusion. BP and infarct volume were significantly correlated, and showed that differences in BP accounted for 15.4% of the variability in size of infarcts. Mortality was 9%, similar to other models of distal MCA occlusion. The advantages of the present model are the reproducibility and consistency of cortical infarcts, the relative ease of the surgical procedure, and that the photothrombosis is less invasive than mechanical occlusion. The model more realistically simulates the pathophysiological events of a thrombotic stroke in humans. Supported by NS-05820, NS-22503 and NS-23244.
NEUROPATHOLOGICAL CORRELATES OF MAGNETIC RESONANCE IMAGES OF ISCHEMIC DAMAGE IN A BABOON MODEL OF CEREBRAL ISCHEMIA. C.A. Parada, L.E. Mankins, J.W. Mathews, P.S. Barker, S.L. Blackburn, and R.N. Bryan. Neuropathology Laboratory, Department of Pathology, and Division of Neuropathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

A baboon model of regional ischemia of the middle cerebral artery was developed to evaluate cerebral metabolism in the early postischemic period with magnetic resonance imaging (MRI), proton magnetic resonance spectroscopy (PMRS), and neurocytology with histological and immunocytochemical analysis (i.e., phosphorylated and nonphosphorylated neurofilaments, MAP2, calbindin, and parvalbumin) to evaluate morphological changes. Abnormal signal intensity was seen as early as 30 minutes with MRI, 3.12 hours with 12 weighted, 3.32 hours with TI weighted, and 4.92 hours with spin density images. Neuropathological evaluation 18 hours after occlusion demonstrated regional dysruption of myelin and neuronal necrosis that correlated with MRI signal changes. However, additional neuronal changes, such as immunoreactivity for calbindin and phosphorylated neurofilaments, and loss of immunoreactivity for MAP2 were detected in a greater area than shown by MRI. In conclusion, MRI appeared to detect tissue necrosis but was less successful in detecting earlier changes of cell damage.

EARLY NEURONAL CHANGES IN THE GLOBAL CEREBRAL ISCHEMIA. K. Kawai, K. Nitsche, C. Rueßler, J. Lohr, N. Sato, T.S. Nowak Jr., and J. Klaatsch, Lab of Neuropathology and Neuroanatomical Sciences, NIH, NINDS, Bethesda, MD 20892.

In elucidation of the pathophysiology of global cerebral ischemia the early neuronal changes were studied in subcriminal 10 minutes cardiac arrest by compression of major cardiac vessels. The early neuronal changes were manifest in animals sacrificed 15 minutes after reperfusion and consisted in appearance of a clear peripheral zones in the cytoplasm. The presence of well preserved mitochondria. The immunostaining for parvalbumin showed disappearance of specific staining of middle portion of the NRT in animals sacrificed 3 hours after reperfusion. Our observations suggest a possibility that early changes in GABAergic neurons may provide a period of diastasis and thus contribute to an excitatory damage in regions connected by GABAergic circuitry.

HEAT SHOCK AND TUBULIN mRNA IN POSTISCHEMIC BRAIN. K. Kumar, B.V. Madhukar. Departments of Pathology, and Pediatrics, Michigan State Univ, E. Lansing, MI 48824.

Recent evidence suggests that while total protein synthesis is reduced following brain ischemia, the expression of a family of heat shock proteins may actually be enhanced in the postischemic (PI) brain during reperfusion. The expression of HSP-70 in PI brain, and to compare it with that of tubulin, a protein abundant in neural tissue, ischemia was produced in gerbils by bilateral carotid artery occlusion for 10 minutes and the carotid was reopened for 6 and 4 hours. The forebrains and consisted in appearance of a clear peripheral zones in the cytoplasm of predominantly GABAergic neurons. After one hour these peripheral zones appeared to compartmentalize into individual clear vacuoles and they were shown to contain normal appearing endoplasmic reticulum. The other neuronal populations showing similar changes were: n. dorsalis medialis thalami, n. centralis amygdalae, pars reticulata substantiae nigra, n. ventralis intermedius, and n. posterior thalami (NRT). The other neuronal populations showing similar changes were: n. dorsalis medialis thalami, n. centralis amygdalae, pars reticulata substantiae nigra, and hypothalamic area. Preliminary immunohistochemical observations on the NRT neurons revealed peripheral vacuoles surrounded frequently by membranes, compatible with endoplasmic reticulum origin and the presence of well preserved mitochondria. The immunostaining for parvalbumin showed disappearance of specific staining of middle portion of the NRT in animals sacrificed 3 hours after reperfusion. Our observations suggest a possibility that early changes in GABAergic neurons may provide a period of diastasis and thus contribute to an excitatory damage in regions connected by GABAergic circuitry.

TEMPORAL RELATIONSHIP BETWEEN REACTIVE GLIOIDIS AND NEURONAL NECROSIS IN POST-ISCHEMIC HIPPOCAMPUS. C. DelRio and I.A. Halaby. Dept. of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642.

Since astrocytes may protect the brain from ischemia, this study tests the hypothesis that reactive astrocytosis develops in areas of CA1 neurons of post-ischemic hippocampal where cell death is characterized by delayed delayed between 3 and 7 days. Cerebral ischemia was produced by the 4-vessel occlusion rat model with reperfusion of 1 to 14 days, producing a 12 min or moderate-severe (10 min) CA1 necrosis. Astrocyte reactivity was evaluated by changes in glial fibrillary acidic protein (GFAP) and vimentin (Vim) and by incorporation of bromodeoxyuridine (BrdU) given 1 and 2 hours prior to sacrifice. Normal control (NC) astrocytes were GFAP+, Vim−, and BrdU−. Increased GFAP preceded neuronal necrosis in 5 of 10 rats between 1 and 3 days after 10 min ischemia and in 5 of 6 rats between 3 and 7 days after 2 min ischemia. Vim− astrocytes and BrdU+ cells were present following 10 minutes of ischemia but rare after 2 minutes of ischemia. These results show that glial reactivity is proportional to the severity of neuronal necrosis. Furthermore, they indicate that reactive astrocytosis may precede the development of neuronal necrosis in vulnerable CA1 neurons, especially when the ischemic insult is prolonged. Supported by NS03464-30, NIMHDS.


Changes at the level of gene expression are becoming increasingly important in understanding the complex changes that occur following global ischemia. In order to determine whether injury alters the expression of gene products potentially related to cellular homeostasis, we have measured changes in the expression of three mRNA species: calbindin-D28K, grp78, and grp94, and hsp72 increased in the hippocampus following seizures. Peak expression occurred 6-12 hrs after administration of KA, and returned to baseline by 24 hrs. Similar changes in all 4 transcripts were seen in coronal sections from fresh-frozen brain sections. HSP70 mRNA was isolated from specific CNS regions. Northern blots were probed with probes for calbindin-D28K, the 78, 84, and 94 kDa glucose-regulated proteins (grp78, grp84), the inducible 112 kDa heat shock protein (hsp72), and a control probe for the 18S ribosomal subunit. Results show that mRNA for calbindin-D28K, grp78, grp84, and hsp72 increased in the hippocampus following seizures. Peak expression occurred 6-12 hrs after administration of KA, and returned to baseline by 24 hrs. Similar changes in all 4 transcripts were seen in coronal sections from fresh-frozen brain sections. HSP70 mRNA was isolated from specific CNS regions. Northern blots were probed with probes for calbindin-D28K, the 78, 84, and 94 kDa glucose-regulated proteins (grp78, grp84), the inducible 112 kDa heat shock protein (hsp72), and a control probe for the 18S ribosomal subunit. Results show that mRNA for calbindin-D28K, grp78, grp84, and hsp72 increased in the hippocampus following seizures. Peak expression occurred 6-12 hrs after administration of KA, and returned to baseline by 24 hrs. These results extend the repertoire of known changes in mRNA expression following CNS injury. The increases in hsp72 and grp84 indicate the occurrence of a generalized stress response. Furthermore, the evidence that grp78 and grp84 are induced by calcium ionophores in vitro, and the potential role of calbindin-D28K in buffering cytoplasmic calcium, the changes observed in this study may represent a cellular response to the perturbed calcium homeostasis that is common to all 3 forms of injury.

HEAT-SHOCK PROTEIN (HSP70) EXPRESSION IN FOCAL BRAIN INJURY. P.J. Lindberg, T.S. Nowak Jr., A.-L. Ricks, J.H. Allenheck, Dept. of Neurol., USUHS and TMINDS, NIH, Bethesda, MD 20814.

Global ischemic or hyperthermic brain damage alters gene expression including production of mammalian heat shock proteins. We have hypothesized that HSP70 expression indicates an area at risk in focal brain injury. Progressive cortical damage was produced by focal laser pulse in rats (n=14). Double-labeling fluorescence technique (Evans-Blue albumin, FITC-dextran, i.v.) was used to map and measure BBB-damage and microvascular perfusion from fresh-frozen brain sections. HSP70 mRNA was localized by in situ hybridization. While absent at 30 min after injury, expression took place by 2 hrs in a ~300 μm wide rim surrounding an area of expanding BBB-damage. HSP70 mRNA was obtained from fixed sections by reverse transcription from fresh-frozen brain sections. HSP70 mRNA was localized by in situ hybridization. While absent at 30 min after injury, expression took place by 2 hrs in a ~300 μm wide rim surrounding an area of expanding BBB-damage. HSP70 mRNA was localized by in situ hybridization. While absent at 30 min after injury, expression took place by 2 hrs in a ~300 μm wide rim surrounding an area of expanding BBB-damage. HSP70 mRNA was localized by in situ hybridization.
426.17 ZINC INDUCTION OF HSP72 AND CELL DEATH IN PRIMARY Astrocyte CULTURE. R.A. Swanston and E.R. Vaisberg. Dept. of Physiology, UCSD. San Diego, CA 92103. Zinc is a potent inducer of the 72kd heat shock protein (HSP72), and extracellular zinc concentrations may be greatly elevated in brain during pathologic conditions. The present study examines the effect of zinc on HSP72 expression in primary rat cortical astrocyte cultures. Astrocytes were grown to confluency and exposed to zinc chloride in Earle's buffered salt solution. HSP72 expression was induced by incubation with 40μM zinc for 4 hours or 1mM for 15 minutes. Expression occurred in a patchy distribution with HSP72-positive astrocytes frequently forming the margin between dead and viable cells, similar to the pattern seen after focal ischemia in vivo. Exposure to zinc at higher levels, 100μM for 4 hours or 500μM for 1 hour, caused greater than 90% cell death. Elevated extracellular zinc concentrations may contribute to HSP72 induction in brain.


Cerebral ischemia induces the expression of stress proteins, which may limit the extent of cellular injury. To test the hypothesis that induction of stress proteins may limit the regional extent of injury, a new model of focal ischemia was developed. In this model, the MCA was occluded for 24 hr following a 10 min occlusion of the distal middle cerebral artery in Wistar rats. HSP-70 mRNA was measured with a 30-mer oligonucleotide probe using in situ hybridization. Adjacent sections were stained with acid fuchsin and thionin to locate injured neurons. The regional relationship between HSP-70 mRNA and neuronal injury was similar at 4 hr and 24 hr. In all animals, the expression of HSP-70 mRNA encompassed a wider area of cortex than that undergoing infarction. Fluorescent, high levels of HSP-70 mRNA occurred peripheral to the area of infarction. Further, the core of infarction occasionally showed background levels of HSP-70 mRNA. Thus, the expression of HSP-70 mRNA in regions bordering tissue injury is consistent with the hypothesis that induction of stress proteins may limit the regional extent of injury.


Reperfusion following 60 min of focal ischemia induces strong expression of heat shock protein-70 (HSP-70) mRNA in neocortex, striatum, and hippocampus (Neurosci Abstr 16:938, 1990). The objective of the present study was to determine the regional expression of HSP-70 mRNA following focal ischemia of varying duration. Focal ischemia was produced in Wistar rats by transient occlusion of the distal middle cerebral artery (MCA) and both carotid arteries for 5, 10, or 20 min, followed by reperfusion for 2 hr. Regional levels of HSP-70 mRNA were measured using in situ hybridization, employing a 30-mer oligonucleotide probe labeled with S-35. Following 5 min of focal ischemia, mild expression of HSP-70 mRNA was detectable in the outer layers of the MCA zone of neocortex. A 10-min insult triggered moderate expression in the outer and inner layers of the MCA neocortex. A 20-min insult produced stronger expression throughout the MCA neocortex and also in adjacent cortex and lateral striatum. Thus, lengthening the duration of focal ischemia not only increased the intensity of expression, but also increased the number of regions expressing HSP-70 mRNA.


To test whether the 72kd heat shock protein (HSP72) protects CA1 hippocampal neurons against severe ischemia, we induced HSP72 synthesis in rats with mild ischemia and later to ischemia that is normally lethal to most CA1 neurons. Adult rats were exposed to 2 min of 4-vessel occlusion (4-VO) ischemia (Pulcinelli and Duffly, J Neurochem, 40:1500, 1983) and then at 6 hr or 48 hr they were made ischemic for 10 min. Separate groups were exposed to 2 or 10 min of 4-VO alone. The animals were killed 5 days later by perfusion-fixation. Stained coronal (7 μm) sections were examined with the light microscope. CA1 damage was graded by an observer on a scale of 0 (normal) to 3 (maximal). Separate rats were subjected to 2 min of 4-VO ischemia, killed by perfusion-fixation at 6 hr (n=3) or 48 hr (n=3), and HSP72 in the CA1 zone was measured with a monoclonal antibody and the avidin-biotin-peroxidase method. MEAN GRADE CA1 HIPPOCAMPAL DAMAGE

<table>
<thead>
<tr>
<th>Condition</th>
<th>CA1 Damage</th>
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<tr>
<td>2 min + 10 min</td>
<td>2.7 ± 0.5</td>
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<tr>
<td>10 min</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>(n=9)</td>
<td>(n=11)</td>
</tr>
<tr>
<td>(n=9)</td>
<td>(n=10)</td>
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*Kruskal Wallis plus Mann Whitney U Test 0.025 < p < 0.05 Immunoactivity to HSP72 after 2 min of ischemia was barely detectable at 6 hr but prominent at 48 hr. Two min of ischemia followed by 10 min of ischemia at 48 hr, but not at 6 hr, significantly decreased CA1 damage compared to ischemia alone. The ischemic tolerance induced by 2 min of sublethal ischemia may result from the induction of HSP72.


Excessive release of glutamate contributes to ischemic brain injury. Adenosine is thought to be an endogenous neuroprotective modulator. The aim of this study is to measure the extracellular changes of glutamate and adenosine in cerebral cortex following ischemia-reperfusion using a stroke model with irreversible ischemia induced to the right MCA cortex. The extracellular amino acids and purine metabolites were recovered from the extracellular fluid before, during ischemia and after reperfusion using microdialysis technique and analyzed by high performance liquid chromatography. Glutamate caused a 30-40% reduction of 20-40%. The increase in extracellular adenosine levels was measured using high performance liquid chromatography. The adenosine concentration increased during ischemia and remained around 0.25 μM for at least 2 hr. Dipyridamole (10μM) both slightly but significantly increased adenosine levels at 60 min in hypoxic conditions did not significantly change adenosine levels from their control over 2 1/2 hr.


Adenosine levels were measured, using absorbance HPLC, in aliquots taken at 30 min intervals from static chambers of 2 ml volume each containing 4 hippocampal slices. Slices were kept on a net at an interface between the physiological saline and the humidified atmosphere at a temp of 33-34°C. A recovery period of 1 hr, fresh medium was placed in the chambers. In 95%O2/5%CO2 basal adenosine levels remained around 0.25 μM at least 21/2 hours. Typical population spikes could be evoked. During hypoxic conditions (95%N2) adenosine levels rose to a new steady-state value of 1.35 μM by 60 min. Removing glucose from the medium during normoxic or hypoxic conditions did not significantly change adenosine levels from their control over 2 1/2 hr.

The adenosine agonist CHA (10μM) and the antagonist 8-CPT (10μM) both slightly but significantly increased adenosine levels at 60 min in hypoxia. The adenosine transport blocker, dipridamole (200μM), had no effect on basal adenosine but doubled extracellular adenosine levels above hypoxia alone. Inclusion of the sugar transport inhibitor, phloretin, (1 mM), increased adenosine six-fold compared to normoxia alone and quadded adenosine levels from hypoxia alone. Experiments attempting to demonstrate a negative feedback exerted by adenosine on its own levels were equivocal. Glucose-free medium may be ineffective because of glucose remaining in the slice as philin greatly increased adenosine. Blocking adenosine transport appears to increase extracellular adenosine but dipridamole, like phloretin, can reduce sugar transport.

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Ischemia is a powerful stimulus for excitotoxic amino acid release from the brain. Adenosine levels are also elevated during cerebral ischemia and purines are cerebroprotective against ischemic and excitotoxic cellular damage. Adenosine analogs are able to reduce glutamate and aspartate release from nerve terminals, we examined the ability of two such analogs, N6-cyclopentyl adenosine (CPA) and N-ethylcarboxamido adenosine (NECA) to depress amino acid release from the ischemic rat cerebral cortex. Using the cortical cup technique and in vitro superfusates of artificial CSF were obtained prior to, during, and following a 20 min period of four vessel occlusion. CPA \(10^{-9}\) to \(10^{-5}\) M and NECA \(10^{-6}\) M had no significant effect on release. The specific \(A_2\) receptor antagonist, 1,3-dipropyl-8-cyclopentyl-xanthine \(10^{-7}\) M also reduced ischemia-evoked release of these amino acids. Adenosine depresses transmitter amino acid release via high affinity \(A_2\) receptors and coactivation of lower affinity \(A_2\) receptors may block the \(A_1\) response.


Calcium and neurotransmitter release have been implicated in hypoxia/ischemia-induced damage. To define the molecular basis of hypoxia-induced alterations in a cellular system, viability, ATP, cytosolic free calcium (\([Ca^{2+}]_c\) ) and dopamine release were examined during early stages (5 minutes) of chemical hypoxia (i.e. KCN) in PC12 cells. High concentrations of KCN (0.5 mM) significantly altered these variables only when glucose was omitted from the media. The response of these same factors to lower concentrations (0.005 and 0.05 mM KCN) was examined in the absence of glucose. ATP levels did not change with 0.005 mM-KCN, but declined by 57% and 84% of control at the two higher concentrations. Similarly, \([Ca^{2+}]_c\) was unchanged at the 0.005 mM-KCN, but increased 2- and 5-fold of the control at the two higher concentrations. Depolarization increased \([Ca^{2+}]_c\) and the values in the hypoxic cells after depolarization increased \([Ca^{2+}]_c\) to 2- and 5-fold of the control at the two higher concentrations. Depolarization increased \([Ca^{2+}]_c\) and the values in the hypoxic cells after depolarization were elevated. The increasing KCN concentrations elevated dopamine in the media under resting conditions (43, 134 and 296% of control) and after K⁺-stimulation (5, 52 and 81% of the K⁺-stimulated control). PC12 cells are a convenient cellular system in which to establish the mechanism by which hypoxia alters dopamine release.


Oxygen free radicals have been implicated in the pathogenesis of brain edema and neuronal cell death in cerebral ischemia. Our previous studies have demonstrated that liposome-entrapped SOD significantly reduced the infarct size after middle cerebral artery occlusion (MCAO) in rats. We have confirmed that infarction volume in transgenic mice, which are genetically modified to overexpress human Cu/Zn-SOD (hSOD-1), was significantly reduced following permanent MCAO (p<0.05). The purpose of present study was to determine the effectiveness of SOD in transgenic mice as compared to normal diploid transgenic mice carrying the hSOD-1 gene were produced as described by Epstein et al (1987). SOD1 activity was identified by native gel electrophoresis followed by staining with nitroblue tetrazolium. All mice weighing 30-35 grams were anesthetized with chloral hydrate (300 mg/kg) injected intraperitoneally, followed by introducing a 5-0 rounded tip suture into the left internal carotid artery 11 mm past the site of the carotid canal bifurcation. Reperfusion was performed by withdrawal of the 3 mC/3AAC followed by 3 hrs reperfusion. Infarction area, measured by image analysis after 2,3,5-phenylindolyltetrazolium chloride staining, was smaller in transgenic mice than wild-type nontransgenic mice (5.7, 4.5, and 4.6 mm distal to the frontal pole) 13,219, 22, 1285, 19, 0023, and 6.9174, respectively. The neurologic deficit scale (0-4) in transgenic mice was lower than that in nontransgenic mice (p<0.05). Our results suggest that superoxide radicals play an important role in the pathogenesis of ischemic infarction in temporary MCAO and reperfusion. (Supported by AG09238, NS-14543, NS-25372, and NS-22202).
427.9 NEUROPROTECTIVE EFFECTS OF GM1 TREATMENT FOLLOWING CNS ISCHEMIA INVOLVE RESTORATION OF CELLULAR MEMBRANE LIPID METABOLISM AND DEFENCE AGAINST OXY-RADICAL TOXICITY. 


Several studies using both in vivo and in vitro models of CNS ischemia have shown that GM1 treatment reduces intracellular edema & metabolic & glutamate release thereby indicating a protective & restorative effect on cellular plas- 
membrane function (1). This study was undertaken to determine if the precise mechanism of GM1 efficacy is unclear. Ischemia initiates membrane fatty acid release. Following reperfusion, the metabolism of released fatty acids together with increased cellular membrane metabolism generate oxygen radicals which contribute to additional membrane damage by lipid peroxidation, inactivates vital cellular enzymes & the expression of certain vital genes. Using a model of focal cortical ischemia in rat (1) we find that acute GM1 ganglioside treatment (10 mg/kg, i. p. 24/4 hrs after ischemia) restored membrane lipid metabolism (increased fatty acid acyla-
tion in peri-infarct areas & partially restored metabolism in the primary is-
cheric area at 2 weeks after the ischemic episode. There was also an increase in the capacity of the oxy-radical defense system evidenced by an increased antioxidant in the already elevated levels of superoxide dismutase, glutathione peroxidase & catalase. This increase by GM1 was sustained for up to 6 weeks. We hypothesize that the neuroprotective effects of GM1 are a result of its effect on those processes which restore and maintain plasma membrane fatty acid oxidation & distribution.

This work was supported in part by a grant from NINDS NS-25856 and a research grant from the FIDIA Research Foundation.

1. Karpiak et al. Temporal changes in edema, Na+, K+ & Ca+ in focal cerebral stroke. GM1 ganglio-


427.10 POST-ISCHEMIC DETECTION OF HYDOXYL RADICALS IN G EBIL BRAIN: ATTENUATION BY THE 21-AMINOSTEROID TRILAZAD METISLYATE (U-74006F). P.R. Andreus*, E.D. Hail, J.A. Atkians, C.M. Williams*, and P.V. Zeitlinger. CNS Dis-
Res. The Upjohn Co., Kalamazoo, MI 49001.

During ischemia and particularly upon reperfusion, oxygen free radicals are formed. An important free radical species is the hydroxyl radical OH•. Salicylate can be used as a marker for the production of OH• as salicylate reacts with it to form 2,3- & 2,5-
dihydroxy benzoic acid (DHBA). To investigate whether pretreat-
ment with the 21-amino-steroid antioxidant U-74006F can reduce brain post-ischemic formation of OH•, as measured by DHBA formation, gerbils were given i.p. doses of either vehicle or U-74006F (10 mg/kg) ten min. before bilateral carotid occlusion (BCO). Following the 10 min BCO, gerbils were allowed reperfusion times of 1, 5 and 15 min. Salicylate was administered i.p. 10 min before sacrificing the animals. Brain hemisections, hippocampus or cortex were removed and immediately frozen. Tissues were homogenized in ethanol (1% ascorbate) and analyzed by HPLC. Data were expressed as the ratio of DHBA to salicylate measured. Results indicated the 2,5-DHBA/salicylate ratio was indeed increased at 1 min reperfusion; pretreatment with U-74006F at-
tenuated DHBA production at all time points.

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The effect of nerve growth factor on the delayed neuronal death after microtubulin associated protein 2 (MAP2) in its protective mechanism of neuronal death (DND) and the involvement of neurofilament (NF) and Laboratories2.

Neurobiology, Kyoto Pharmaceutical University1, Shionogi Research Kimura1, T. Taniguchi1, O. Ohara2 . Department of Neurosurgery, ischemia. K. Tanaka, Y. Yonekawa, T Tsukahara, N. Ogata, Y. Kaku, T.

EXPRESSION OF MAP2 mRNAs IN RAT AND GERBIL BRAIN

DND were investigated. Wister rats were divided into following three groups; Group 1; 8 min transient forebrain ischemia. Group 2; intraventricular administration of 2 μg of 2.5S NGF intraventricular administration of artificial cerebrospinal fluid (CSF) and die. Group 3; intraventricular administration of 2 μg of 2.5S NGF and by immunohistochemical staining with anti-NF200 and anti-MAP2 antibody followed by an 125I-labeled secondary antibody. The distribution of CaM-KII between cytosolic and particulate fraction. A time dependent decrease occurred in the amount of CaM-KII enzyme in the CA1 after the addition of Ca2+ and calmodulin. This process was partially blocked by specific inhibitors of CaM-KII and the phosphatase inhibitor okadaic acid.

TRANSLLOCATION OF Ca2+ CALMODULIN-DEPENDENT PROTEIN KINASE II AFTER FOREBRAIN ISCHEMIA

In transient forebrain ischemia induced by brain vessel occlusion in rats, resulted in depressed activity of both CaM-KII and Ca2+-phospholipid-dependent kinase while no changes were detected in the activity of CaM-dependent kinase, as measured in both cerebral cortex and hippocampus. The decrease in CaM-KII activity, measured by phosphorylation of synthetic peptide substrate, coincided with translocation of the enzyme from the cytosol to the particulate fraction, determined using a CaM-KII specific monoclonal antibody followed by an 125I-labeled secondary antibody. The translocation and transactivation of CaM-KII was fully reversible following 2 hr of reperfusion of animals exposed to 5 min of ischemia. In contrast, the translocation and inactivation of CaM-KII following 20 min of ischemia was not completely reversible with reperfusion. The mechanism(s) responsible for down regulation and/or translocation of CaM-KII is unknown. Phosphorylation due to increasing intracellular Ca2+ following ischemia is one possible step in this pathway. In preliminary experiments to test this hypothesis, we phosphorylated crude homogenates of rat cerebral cortex and examined changes in the distribution of CaM-KII between cytosolic and particulate fraction. A time dependent decrease occurred in the amount of CaM-KII enzyme in the CA1 after the addition of Ca2+ and calmodulin. This process was partially blocked by specific inhibitors of CaM-KII and the phosphatase inhibitor okadaic acid.

DISAPPEARANCE OF BRAIN Ca2+/CALMODULIN-DEPENDENT PROTEIN KINASE II AFTER BRIEF ISCHEMIA

Several studies have suggested that ischemia produces neuronal cell death by the elevation of intracellular calcium which activates calpain, resulting in extensive proteolysis. In this paper, we examined the effects of forebrain ischemia in gerbils on Ca2+/calmodulin-dependent protein kinase II (CaM-KII) by both kinase assay activities and Western blot analysis. In cortex and hippocampus, cytosolic CaM-KII was completely lost within 2.5 min of ischemia. Particulate CaM-KII II was more stable and decreased about 40% after 10 min of ischemia followed by 2 hrs reperfusion. CaM-KII in cerebellum, which does not become ischemic, was not affected. The rapid loss of forebrain CaM-KII II was quite specific since hippocampal cytosolic CaM-kinase and protein kinase C were not affected. These data strongly suggest that very brief ischemia causes immediate proteolysis of soluble CaM kinase II which would be deleterious since CaM-KII II modulates numerous neuronal cell functions.

ACTIVATION OF PROTEIN KINASE C BY H2O2 IN UC11MG ASTROCYTES

In vitro rat hippocampal slices were equilibrated with artificial cerebrospinal fluid (ACSF) containing 10 mM manitol. Ouabain (100 μM) was then added to the manitol-containing ACSF to provoke a spreading depression (SD) - like depolarization. Ouabain was perfused for as long as 30 minutes, and no SD-like event was observed. Moreover, population spike (PS) in the CA1 region was still present at the end of this time. By contrast, in separate experiments neither ATP (50 μM) nor kynurenic acid (100 μM) prevented ouabain-induced SD-like depolarization and the consequent disappearance of PS.

In other experiments, perfusion with ACSF that was made supersaturated with artificial cerebrospinal fluid (ACSF) containing 10 mM manitol. Ouabain (100 μM) was then added to the manitol-containing ACSF to provoke a spreading depression (SD) - like depolarization. Ouabain was perfused for as long as 30 minutes, and no SD-like event was observed. Moreover, population spike (PS) in the CA1 region was still present at the end of this time. By contrast, in separate experiments neither ATP (50 μM) nor kynurenic acid (100 μM) prevented ouabain-induced SD-like depolarization and the consequent disappearance of PS.

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Humans explore their world with saccadic eye movements. These are used in orienting, looking, searching, tracking, and perhaps dreaming. The processing for saccadic eye movements includes analysis of what in the three targets are in space, the cognitive decisions of choosing where to make a saccade, and the motor programming to move the eyes. The innervation of the oculomotor system is visible to psychophysical, physiological, and theoretical study, and we will discuss the concepts and controversies surrounding the neural mechanisms of this, the best understood sensorimotor system in man.

B. Deubel will discuss "The sensory stimulus". Optican will discuss "The superior colliculus and sensorimotor transformation". Goldberg will speak about "Cortical mechanisms for spatial accuracy". Anderson will conclude with "Coordinate transformations in posterior parietal cortex".

431.1
The Organization of Cytochrome-Oxidase Blobs in Cat Visual Cortex. K. M. Murphy*, H. C. Van Sluyters*, D. G. Jones*. Departments of Psychology and Electrical Engineering, McGill University, Montreal PQ H3A 1B1, School of Optometry, University of California, Berkeley, CA 94720.

The primary visual cortex is parcellated into modules that have been defined using both physiological and anatomical techniques. Quite possibly the most striking demonstration of cortical modularity is that of the blobs of higher cytochrome-oxidase activity in cat visual cortex. In an effort to overcome these problems, we have begun to study using both physiological and anatomical techniques. Quite possibly the most striking demonstration of cortical modularity is that of the blobs of higher cytochrome-oxidase activity in cat visual cortex. We have shown that activity-dependent sprouting of motor axons in striate cortex involves regulation of adhesion molecules via enhanced expression of an unusual cell surface carbohydrate. Recent investigations in the cortex, ultraviolet eye ganglion neurons grown in culture, have shown that activity-dependent sprouting of motor axons in striate cortex involves regulation of adhesion molecules via enhanced expression of an unusual cell surface carbohydrate. Recent investigations in the cortex, ultraviolet eye ganglion neurons grown in culture, have shown that activity-dependent sprouting of motor axons in striate cortex involves regulation of adhesion molecules via enhanced expression of an unusual cell surface carbohydrate.
341.5

LEARNING SENSORIL MAPS IN THE VISUAL CORTEX


Orientation specificity in the visual cortex is organized in an ordered manner and changes gradually so that adjacent cells have (on average) similar preferred orientations. The preferred orientations form an interleaved hexagonal structure and at several points (singularities) all orientations meet. The average orientation tuning strength is low at such singularities. This local coupling suggests that preferred orientation and orientation tuning strength may be linked globally both being a function of the cortical surface coordinates. We demonstrate that such a link actually exists and that it can be described by a single analytic expression. In good accordance, the map of the orientation tuning strength is obtained from the map of the preferred orientations by Fourier transformation methods. We found good correspondence between the computed maps and the experimentally measured maps obtained from optical imaging of intrinsic signals (Bonhoeffer & Grinvald, Neurons. Abs., 1990 and unpublished data). This supports the view that a common design principle underlies the maps for preferred orientations and orientation tuning strengths and we expect this result to facilitate progress towards a more unified theory of the development of visual cortex. We thank Drs. Grinvald & Bonhoeffer for sharing with us their unpublished data in digital form.

341.6

OPTICAL IMAGING REVEALS PREFERENTIAL LABELING OF CYTOCHROME OXIDASE-RICH REGIONS IN RESPONSE TO COLOR STIMULI IN AREA V2 OF MACAQUE MONKEY

C. E. Landisman, A. Grinvald, D. Y. Ts'o. Laboratory of Neurobiology, The Rockefeller University, NY, NY, 10021 and The Weizmann Institute, Rehovot, Israel.

In vivo optical imaging of the cytochrome oxidase bios when using the intrinsic (no dye) optical signals has been performed by localizing the centers of monocular, duration of stimulation with a moving retinotopic grating. We now report that optical imaging maps have demonstrated cortical regions that yield a notably stronger response to low spatial frequency isoluminant chromatic gratings than to their achromatic counterparts. These patches of strong response to chromatic gratings coincide with the cytochrome-oxidase rich regions. Results suggest, however, that the segregation of responses is not complete. There are residual responses of the interblob regions to the chromatic stimuli and of the blobs to luminance stimuli. Indeed with very low spatial frequency stimuli, striate cortex in general was more responsive to chromatic than luminance stimuli in V2, isoluminant color stimuli preferentially labeled color-selective subcompartments of the stripes.

Optical imaging during standing versus flashing visual stimuli has also shown preferential labeling in V1 and V2. Preliminary results suggest that interblob regions and the thick stripes are less responsive to standing stimuli than flashing stimuli. Using our ability to localize the blobs in vivo with optical imaging, we have begun to target specific blobs with electrical recordings to explore the relationship between the optical responses to color stimuli and the electrophysiological assessment of receptive field properties. (Supported by EY08240 and the Whitaker Foundation).

341.7

ORIENTATION SELECTIVITY OF AND INTERACTIONS BETWEEN COLOR AND DISPARITY SUBCOMPARTMENTS IN AREA V2 OF MACAQUE MONKEY

D. Y. Ts'o, C. D. Gilbert, T. N. Wiesel. Laboratory of Neurobiology, The Rockefeller University, NY, 10021.

By combining optical imaging maps of V2 with electrophysiology, we have studied the relationship between the stripes that contain disparity sensitive cells and stripes that contain color selective cells. Both cytochrome oxidase history and the optical imaging maps have suggested that disparity components in V2 are strictly organized with a retinotopic character. This is in contrast to color components, all within a single histologically-defined "stripe". Systematic electrode penetrations across these regions reveal a gradual progression of receptive field properties from cells with sharp disparity tuning and no color selectivity, to cells having both disparity tuning and color selectivity, to color selective cells with no disparity tuning. After injections of retrograde tracers in V2, receptive-field maps reveal that the disparity sensitive cells are organized in thick stripes that contain disparity sensitive cells and the color selective cells are organized in thin stripes. The thick stripes are oriented perpendicular to both the thick stripes of chromatic cells and the interstripes of achromatic cells. The thin stripes are oriented parallel to both the thin stripes of achromatic cells and the interstripes of chromatic cells. Optical imaging during standing versus flashing visual stimuli has also shown preferential labeling in V1 and V2. Preliminary results suggest that interblob regions and the thick stripes are less responsive to standing stimuli than flashing stimuli. Using our ability to localize the blobs in vivo with optical imaging, we have begun to target specific blobs with electrical recordings to explore the relationship between the optical responses to color stimuli and the electrophysiological assessment of receptive field properties. (Supported by EY08240 and the Whitaker Foundation).

341.8

CHARACTERIZATION OF DYNAMIC PATTERNS OF CORTICAL ACTIVITY BY A SMALL NUMBER OF PRINCIPAL COMPONENTS

D. Shoham, S. Ullman and A. Grinvald. Weizmann Institute, Rockefeller University and MIT.

In previous years our group (Arieli et al 88, 90) has used real-time optical imaging to study the spatio-temporal organization of the activity of large neuronal populations in cat visual cortex. The activity of a 3x3mm patch of cortex was imaged by use of an array of 12 detectors. We have observed complex coherent patterns which scanned the cortex at several frequencies. The results were presented as 'brain wave movies'. While these movies appear quite irregular and complex, a closer inspection reveals several recurring spatial patterns.

Here we attempt to measure the level of complexity inherent in those cortical waves by using Principal Component Analysis. In this method the given set of patterns is used to define a base of orthogonal patterns (principal components), which under a specific mathematical criterion is optimal for representing all the data. Each of the original patterns is decomposed into a weighted sum of the principal components. While the complete base of principal components is needed to fully represent the data, a small subset may give a good approximation in some cases where the data exhibit high regularity.

We applied this method to the brain wave movies, using all the movie frames as the data set. Surprisingly, we found that as few as 3-10 principal components (out of 120) were sufficient to approximate an entire experimental movie with an accuracy of 80-90%. An examination of the weights of the different components as a function of time revealed that the weight of the first component is proportional to the temporal signal averaged over detectors. We compared the components obtained under different stimulus conditions and found a high degree of similarity. These results suggest that further analyses might be simplified by replacing the data by the first few components. More importantly, they seem to point to a basic low-dimensional underlying cortical activity. (Supported by The Riklis Family Foundation.)

341.9

INTRACORTICAL INTERACTIONS IN CAT VISUAL CORTEX: EVIDENCE FROM POSTSYNAPTIC FIELD POTENTIALS.


The extensive lateral spread of the dendritic and axonal arborization of cortical neurons is not reflected in the functional organization of single unit receptive fields which is retinotopic and columnar. We have found local post synaptic potentials which reflect long-range lateral connectivity. We compared extracellular single-unit responses and local field potentials in area 17 of the cat using a pair of microelectrodes (tip separation, ~300µm). Single-unit receptive fields were plotted with sharp disparity tuning and no color selectivity, to cells having both disparity tuning and color selectivity, to color selective cells with no disparity tuning. After injections of retrograde tracers in pure color or disparity compartments, label is found in border regions containing color disparity cells, up to 6mm away.

Within the disparity zones, cells tended to have vertical or near vertical orientation preference. This result was most pronounced among cells with the sharpest disparity tuning, which were also unresponsive to monocular stimulation. In general, we found two types of regions in V2 containing oriented cells; one in which orientation was organized in a columnar fashion, where the preferred orientation was very uniform and altered very little both for the length of a vertical penetration and for neighboring penetrations. A second type of region of oriented cells exhibited no organization for orientation, but instead the preferred orientation changed rapidly not only for neighboring penetrations, but also for neighboring cells within a single vertical penetration. These two types of organization for orientation were reflected in the optical imaging orientation maps. (Supported by grants EY07968, EY05253, EY08240 and the Whitaker and Rita Allen Foundations).

341.10

EXPERIENCE-DEPENDENT INTRACORTICAL INTERACTIONS IN CAT VISUAL CORTEX: EVIDENCE FROM POSTSYNAPTIC FIELD POTENTIALS


Cortical neurons in area 17 receive intracortical lateral afferents from a large cortical area. Using bipolar microelectrodes, we have shown the presence of large "receptive fields" for post synaptic potentials generated within a small cortical map responsive to retinotopic gratings. (Kitano et al., Invest. Ophthal. Vis. Sci. Suppl., 1991). Using multiinput, non-linear systems analysis, the field potentials generated in normal visual cortex were divided into fast (retinotopic) and slow (non-retinotopic) post synaptic potentials. The latter had a large "receptive field" and showed strong spatial interactions inside this field.

In cats which had one eye lid-sutured for 4 years from the age of one week, no single units were found in area 17 responding to stimulation of the lid-sutured eye, but field potentials were evoked. These field potentials had only one component whose waveforms was similar to the non-retinotopic slow component obtained by stimulation of the normal eye. However, their receptive field was small, retinotopic and did not exhibit spatial interactions. In kittens which had received single-orientation visual experience binocularly, single units in area 17 showed normal receptive-field properties. In contrast, the characteristics of the field potentials were absent or essentially the same as those obtained for stimulation of lid-sutured eye. These findings suggest that normal development of extensive lateral interactions in visual cortex is essential for cortical function. (USPHS Grants BRSR-05981, Core Grant EY-06883)
432.1 MOLECULAR CLONING AND EXPRESSION OF A NOVEL D1 DOPAMINE RECEPTOR WITH A UNIQUE DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM. M. Timko, K.R. Jiang, G. Silveira, P. Falardau, J.A. Ginschin, T-Y. Yang-Feng, B.T. Fremaje Jr. and M.G. Caron. Dept. of Cell Biology, Duke University Medical Center, Durham, NC 27710.

Cloning techniques have now established that five different genes code for dopamine receptor subtypes in the central nervous system. Two D1 receptor subtypes and three D2 related subtypes. Here we report the cloning of a rat gene coding for a novel D1 receptor subtype. The rat gene has an open reading frame of 1425 nucleotides. A cDNA corresponding to the open reading frame encodes a protein of 475 amino acids. This protein has structural features that are conserved in the previously characterized receptor subtypes. The expressed protein binds dopaminergic ligands with a pharmacological profile similar to the previously cloned rat and human D1 dopamine receptors. In conclusion, the D1 receptor represents a new subtype of dopamine receptors: glycosylation sites in the extracellular domains, conserved aspartate residues in the transmembrane domain. This latter transcript was stably encoded receptor protein contains all the essential hallmarks of catecholamine receptors: glycosylation sites in the extracellular domains, conserved aspartate residues in the transmembrane domain. This latter transcript was stably expressed in hippocampus and hypothalamus. In situ hybridization showed that high levels of mRNA are found in distinct layers of hippocampus, the mammalian nuclei and the anterior pre-tectal nuclei. In contrast to the previously cloned D1 receptor, little or no mRNA for this novel receptor was observed in striatum, nucleus accumbens, olfactory tubercle and the fronto-parietal cortex.


Using Polymerase Chain Reaction (PCR) amplification of mRNAs from several areas of rat brain we have shown the occurrence of two shorter transcripts of the dopamine D3 receptor. The shorter variant contains a frame shift at the 3′ untranslated region of the D3 receptor. Treatment of SK-N-MC cells with 10μM dopamine for varying lengths of time caused a 40% increase in D3 mRNA by Northern blot analysis followed subsequently by a rapid decline to 70% of untreated levels by 3 hours and a slower decline to 50% by 12 hours. D3 receptor levels as measured by TPH-2 mRNA binding show a slight rise over one hour followed by a decline to 37% of control by 12 hours. The observed increase in D3 mRNA is probably due to increased synthesis, since nuclear runoff experiments show a two-fold increase in D3 mRNA synthesis in reticulocyte lysates supplemented with dopamine for one hour. D3 mRNA half-life is unchanged in further cells, supporting this hypothesis. The responses of D1 receptor and mRNA to agonist are similar to those found for the β2 receptor and the β3/CC receptor, raising the possibility that this is a general phenomenon associated with G-protein coupled receptors that stimulate adenylyl cyclase.
STABLE TRANSFECTION OF CELL LINES WITH DOPAMINE RECEPTORS


Classically, dopamine receptors have been divided into two subtypes, D1 receptors linked to stimulation of adenyl cyclase with high affinity for SCH-23390, and other benzamides, and D2 receptors linked to inhibition of adenyl cyclase with high affinity for butyrophennones and other neurotoxins. Six subtypes of dopamine receptors have been cloned and sequenced. D1 and D2 receptor-DNA clones have similar coding sequences, and, when expressed in mammalian cells, have the pharmacological profile and second-messenger linkage expected of D1 receptors in striatal homogenates. D2 receptors are similar in coding sequence and possess the pharmacological properties associated with D2 receptors.

The multiplicity of dopamine receptors and the complexity of the central nervous system, the pharmacological properties of these receptors are difficult to resolve in vivo. Selective expression of subtypes of dopamine receptors in mammalian cell lines permits unambiguous analysis of these subtypes.

The genes coding for several dopamine receptors have recently been characterized. The D2, D3, and D4 receptor classes are all recognized by ligands previously considered to be "D2-like." In order to establish a differential labeling of these sites with the available "D2-like" ligands, we first identified the brain regions enriched for mRNA coding for a specific receptor form in situ hybridization histochemistry using [3H]-labeled oligonucleotides. The pharmacological characteristics of the receptor form in each region were determined by direct labeling with [3H]-spiperone, [3H]-raclopride, [3H]-CV 205 522, [3H]-lisdexipridine and [3H]-YM 09151-2. Furthermore, displacement experiments using unlabeled compounds like dopamined, raclopride, clozapine and quinpirole, which present some selectivity for the different "D2-like" receptor classes, were carried out. The results obtained show that by combining selected radioligands with unlabeled compounds experimental conditions can be defined to visualize specifically the different dopamine "D2-like" receptor classes.

RECOVERY OF CENTRAL D1 AND D2 RECEPTORS FOLLOWING ALKYLATION IN YOUNG AND ADULT RAT BRAIN. R.J. Baldessarini, N. Kulé, T. Neveux, J. Anderson Foundation.

We have shown that SN but not striatal injections of the receptor inactivator N-ethyl-

4-aminophenethylspiperone (NAPS), such as the

D-amino-isopropyl derivative of phenylethylamine, which is used for behavioral inactivations in rats. The results obtained show that by combining selected radioligands with unlabeled compounds experimental conditions can be defined to visualize specifically the different dopamine "D2-like" receptor classes.

EFFECT OF SELECTIVE D1 AND D2 DOPAMINE (DA) RECEPTOR INACTIVATION IN SUBSTANTIA NIGRA (SN) ON RESPONSES OF SN DA NEURONS TO I.V. R-JNP.


We have shown that SN but not striatal injections of the receptor inactivator N-ethyl-

4-aminophenethylspiperone (NAPS), such as the

D-amino-isopropyl derivative of phenylethylamine, which is used for behavioral inactivations in rats. The results obtained show that by combining selected radioligands with unlabeled compounds experimental conditions can be defined to visualize specifically the different dopamine "D2-like" receptor classes.

Localisation of D2 DA Receptor mRNA in Primary Olfactory Neurons.

Michael T. Shiple, William T. Nickell, Andrew B. Norman (University of Cincinnati; and Charles Gerfen, Charles C. Emerson, PA 19104).

The glomeruli of the olfactory bulb are densely innervated by dopaminergic (DA) interneurons; the post-synaptic targets of these neurons are unknown. We recently showed that both DA receptor subtypes in the olfactory bulb show a high degree of localization to the glomerular layer. Confocal images of DA receptor mRNA in the olfactory bulb of the rat demonstrate a high degree of localization to the glomerular layer. DA receptor mRNA is also detected in the olfactory bulb of the mouse, confirming the results in the rat. The localization of DA receptor mRNA in the olfactory bulb of the rat is consistent with the post-synaptic targets of DA interneurons being the glomerular layer.

In a series of non-human primate experiments (n=7), the effects of temperature, amphetamine, haloperidol, and reserpine on brain uptake of 123I-IBZM were measured. After i.p. injection of 123I-IBZM (1.9-7.3 mCi), specific brain uptake reached a peak by 100 min and demonstrated a gradual apparent "steady-state" washout over the next 2 hrs. Brain uptake was temperature dependent, with rates of washout of specifically-bound, radiolabeled growth hormone releasing hormone (GHRH) being 1.6%/min at 33°C, 0.8%/min at 30°C and 0.2%/min at 28°C. No significant differences in rCBF were found between any conditions. Improved SEP recovery was found in non-human primates after GHRH treatment, which is consistent with the hypothesis that endogenous dopamine neurotransmitter systems play a role in the pathophysiology of cerebral ischemic injury. The results of this study suggest that receptor imaging may be developed to monitor transmitter turnover.

ISCHEMIA V

TIRILAZAD MESYLATE (U-74006F) ATTENUATES THE ACCUMULATION OF NEUTROPHILIC LEUKOCYTES (NLs) IN ISCHEMIC GERBIL BRAIN. J.A. Ostoven and L.R. Williams, CNS Disease Res., The Upjohn Co., Kalamazoo, MI.

The involvement of NLs was examined in the gerbil unilateral carotid occlusion (UCO) model of transient focal cerebral ischemia (Hall et al., 1988). Frozen cortical sections were processed with cytotoxic oxidase (CO), myeloperoxidase (MPO), and cresyl violet histochemistry. In control gerbils after 3 hr UCO, NLs are clumped within the cerebral vasculature, and are commonly observed crossing the capillaries into the brain parenchyma. After UCO and 24 hr of reperfusion, there is considerable necrosis within the ischemic hemisphere, and numerous CO- and MPO-positive, degranulated NLs are dispersed throughout the necrotic tissue. Computer-assisted image analysis of the lateral cortex indicated an average (± SEM) density of 194 ± 32 (CO) and 202 ± 41 (MPO) cells/mm2 (n=7). In gerbils treated with U-74006F (10 mg/kg i.p.), the density of CO-positive cells was significantly reduced by 66% (p<0.05). The density of MPO-positive cells was reduced 31%. Thus, one mechanism by which U-74006F provides cytoprotection to ischemic brain tissue in vivo is by limiting the accumulation of NLs in tissue at-risk, thus limiting consequent NL-mediated oxidative tissue destruction.

HYPOTHERMIA IN PERMANENT FOCAL ISCHEMIA: EVOKED POTENTIALS, REGIONAL CEREBRAL BLOOD FLOW AND QUANTITATIVE MRI. E.H. Lo and G.K. Steinberg. Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA 94305.

A rabbit model of permanent focal ischemia was used to study the effects of hypothermia on somatosensory evoked potentials (SEP), regional cerebral blood flow (rCBF) and quantitative magnetic resonance (MR) parameters. Three temperature (temporal muscle) groups were used: 37°C, 33°C, and 30°C (n=4 per group). SEPs were measured before and after occlusion. After 4 hrs of permanent focal ischemia, rCBF was measured using radioactive microspheres. Brains were removed immediately afterwards for MRI measurements. Improved SEP recovery was found in the 33°C and 30°C groups compared to the normothermic group (p<0.05). T1 and T2 MRI values of the core ischemic regions were significantly increased in the normothermic group (p<0.05, left vs right hemisphere). In both hypothermic groups, left vs right T1 and T2 values were not significantly different. No significant differences in rCBF were found between any temperature groups. These results demonstrate that hypothermia protects against permanent focal ischemia without altering rCBF.
433.5 POST-ISCHEMIC TREATMENT WITH FELBAMATE REDUCES DELAYED NEURONAL CELL DEATH. G.O. Westfalla, J.M. Adams, and P.H. Schwab, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

Rescuing neurons from delayed cell death should be one of the main goals of post-ischemic therapy. We previously reported that the anti-ischemic action of felbamate is more pronounced if administered immediately after the hypoxic-ischemic insult in reducing delayed neuronal necrosis. We used 7-day-old rat pups subjected to bilateral carotid ligation and exposure to 6% O2 for 1 hour (which had no effect on serum glucose levels). Felbamate (350 mg/kg ip injections or oral administration) raised serum felbamate to 100-150 μg/mL for several hours. Following bilateral carotid ligation and exposure to 6% O2 for 1 hour (which had no effect on serum glucose levels), felbamate (350 mg/kg ip) given immediately (0 hr), 1, 2, or 4 hr after the end of hypoxia significantly reduced the volume of neocortical infarcts by 55%, 30%, 44%, and 24%, respectively. In this model, dentate granule cell death initiated 4-24 hours after insult but displays delayed necrosis by 72 hr. Felbamate given immediately after hypoxia reduced granule cell necrosis by 92% (p<0.001). Single doses of 100 and 200 mg/kg were less effective given after the insult than 300 or 400 mg/kg. These data suggest that felbamate given after a hypoxic-ischemic insult is very effective in rescuing neurons from delayed post-ischemic death.

433.6 LIPOCORTIN-1 INHIBITS CEREBRAL ISCHAEMIA. J.K. Belton*, P. Carey*, D. U. Prichard, and N.J. Rothwell*.

To examine the time course of ischemic injury, we have used organotypic hippocampal culture which preserves synaptic circuitry and regional differences in vulnerability to ischemia. Brief exposure to “ischemia” was achieved with 2-deoxyglucose (2-DG) and potassium cyanide (KCN) to inhibit glycolysis and oxidative metabolism. Propidium iodide fluorescence was used to reveal early membrane injury incurred in the living culture. Staining was most rapid following a 30 minute exposure at high concentrations (5 mM 2-DG, 1 mM KCN), and only started after 2-4 hours of staining with CA1, then involved CA3 with sparing of the dentate gyrus. Histology after 24 hours of recovery demonstrated marked pyknosis of neuronal nuclei in the propidium stained regions. Exposures of either shorter durations (8-15 min) or lower concentrations of 2-DG and KCN produced a pattern of propidium staining which was further delayed (4-6 hours), less intense, and affected only CA1 and then CA4/CA3c, but spared CA3a,b and the dentate gyrus. Histology at 24 hours was largely normal. With longer recovery (3, 5, and 7 days), an increasing severity of pyknosis was noted in the areas showing early propidium staining. The time course and regional distribution of ischemic neuronal degeneration in organotypic culture appears to reproduce that observed in animal models. Membrane injury, as revealed by propidium staining, far precedes histologic degeneration. Furthermore, the pattern of injury is distinct from that produced by brief exposure to NMDA.


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433.8 SEROTONIN MAY MEDIATE PLATELET NEUROTOXICITY. Rajiv Joseph, Chock Tsering, Saul Grunfeld and KMA Welch, Lab. of Experimental Hematology & Strokes, Henry Ford Hospital, Detroit, MI 48202.

Our earlier studies suggested that platelet secretory products have a toxic effect on acetylcholinesterase positive neurons in organotypic spinal cord cultures (Neurology 1991; 41 (Suppl.1):163). Further work was done with added refinements such as use of gel-filtered platelets, platelet membrane and red blood cells as additional controls, and using both rat and human platelets. The results indicated that in our previous study, that platelet secretion has a morphologically demonstrable toxic effect on neurons (p<0.0001). In order to identify a toxic component from among the known platelet secretory products, experiments were carried out exposing the explants to SHT, a major platelet product. Based on the concentration of SHT in whole blood, before and after platelet activation, and the estimated content of platelets in an occlusive thrombus, we calculated that the concentration of SHT around an acute thrombus could well reach 4000 times the concentration in whole blood with the platelets unactivated. We exposed the explant-cultures to varying concentrations of SHT in order to obtain a dose-response curve. There was evidence of neurotoxicity at SHT concentrations of 3.5 μM and higher. The number of surviving neurons in the treated group (SHT 350 μM) was 72% less than in the controls (p<0.0001). Considering that this effect was seen with a SHT concentration (350 μM) that is about one-fourth the calculated concentration that could theoretically be present in the vicinity of an acute thrombus, it appears reasonable to postulate that SHT could significantly contribute to ischemic neuronal injury.


To examine the time course of ischemic injury, we have used organotypic hippocampal culture which preserves synaptic circuitry and regional differences in vulnerability to ischemia. Brief exposure to “ischemia” was achieved with 2-deoxyglucose (2-DG) and potassium cyanide (KCN) to inhibit glycolysis and oxidative metabolism. Propidium iodide fluorescence was used to reveal early membrane injury incurred in the living culture. Staining was most rapid following a 30 minute exposure at high concentrations (5 mM 2-DG, 1 mM KCN), and only started after 2-4 hours of staining with CA1, then involved CA3 with sparing of the dentate gyrus. Histology after 24 hours of recovery demonstrated marked pyknosis of neuronal nuclei in the propidium stained regions. Exposures of either shorter durations (8-15 min) or lower concentrations of 2-DG and KCN produced a pattern of propidium staining which was further delayed (4-6 hours), less intense, and affected only CA1 and then CA4/CA3c, but spared CA3a,b and the dentate gyrus. Histology at 24 hours was largely normal. With longer recovery (3, 5, and 7 days), an increasing severity of pyknosis was noted in the areas showing early propidium staining. The time course and regional distribution of ischemic neuronal degeneration in organotypic culture appears to reproduce that observed in animal models. Membrane injury, as revealed by propidium staining, far precedes histologic degeneration. Furthermore, the pattern of injury is distinct from that produced by brief exposure to NMDA.

433.10 REPERFUSION IN A RAT MODEL OF FOCAL ISCHEMIA. W.S. Selim*, A. Hopkins*, R.C. Crookes, R.A. Batchelor, and W.D. Luck, Departments of Neurosurgery and Anatomy, Case Western Reserve University, Cleveland, OH 44106

Reperfusion following focal ischemia has been shown to be of little benefit in protecting tissue from injury if initiated after 2 h of occlusion. In this report, the metabolic recovery of the focal and perifocal regions was examined at 1 h of reflow either after 1 h [1,1] or 4 h [4,1] of middle cerebral artery (MCA) occlusion in spontaneously hypertensive rats. All rats were given a single injection (4 μl icv) of an active fragment of lipocortin-1 (N-terminal 1-188aa) profoundly reduced (by 71.4 ± 8.5%) the extent of ischemic neuronal damage (infarct volume) assessed at 24h (81.2 ± 15.5 vs 23.2 ± 6.9mm3, n=8, p<0.01). In a separate study, a single injection (4 μl icv), 10 min after MCAo, of neutralizing anti-lipocortin-1 fragment antiserum increased (by 53.2 ± 18.1%) the size of infarct assessed 2h later (22.6 ± 2.4 vs 34.6 ± 4.1mm3, n=10, p<0.05). These findings indicate that lipocortin-1 is an endogenous inhibitor of cerebral ischemia with considerable therapeutic potential.

Permanent MCAO in SHR rats results in ischemic changes characterized by focal pan necrosis, inflammatory cell infiltrate, glialosis, and loss of the necrotic tissue by thirty days post occlusion. We have now characterized changes which occur as a result of the beginning of an inflammatory infiltrate in and around the vasculature. This infiltrate consisted primarily of neutrophils; however, monocytes were present. Infiltration occurred much more quickly and to a greater extent in represented tissues than in permanently occluded tissues, where it began approximately 12 hours post occlusion. Three days after surgery, in addition to the neutrophils, a heavy macrophage infiltrate was present in the reperfused tissues. Macrophages were a minor component of the permanently occluded lesions. Following five days of reperfusion, the necrotic tissue was largely replaced by macrophages, with numerous neutrophils distributed throughout the lesion. In contrast, in the permanently occluded tissues, macrophages were scattered through a loose extracellular matrix and remaining necrotic tissue, and neutrophils were located focially. The data indicate that duration of occlusion affected the size of the lesion, while reperfusion affected the timing, extent and composition of the response that leads to resolution of the necrotic tissue. Histologic and immunohistochemical evidence of these changes will be presented.

CORTICOTROPIN-RELEASING-FACTOR GENE EXPRESSION IS NOT INCREASED AFTER PHARMACOLOGICAL ADRENALECTOMY. Tallie Z. Baram, M.D., PhD and Linda Goldstein, PhD, Dept. of Neurosurgery, University of Southern California; Div. of Neurology, CHLA; Los Angeles, CA 90024

A regimen of twice daily metyrapone injections (100 mg/kg), resulted in pharmacological adrenalectomy of pregnant rats and fetuses in utero: i.e. plasma corticosterone and elevation of plasma ACTH in pregnant and in fetal rats. Toxicity was minimal for days 14-17 of gestation and increased with higher maternal weight and pregnancy progression.

Maternal corticotropin releasing-factor (CRF) messenger RNA abundance increased significantly within 48 hours of metyrapone initiation. No change in CRF gene expression in the paraventricular nucleus of fetuses (days 17-19) was seen, even after 72 hours of the regimen. This is compatible with the independence of CRF gene expression of glucocorticoid feedback in the fetal rat.

DISTRIBUTION OF CRF-BINDING PROTEIN mRNA AND IMMUNOREACTIVITY IN THE RAT BRAIN. E. Potter*, D.P. Reichert*, P.E. Szewczuk and W.W. Vale. The Salk Institute, La Jolla, CA 92037

Initial studies on the structure and tissue distribution of the rat CRF- binding protein (CRF-BP) gene indicated that CRF-BP mRNA was present in the brain. We have used cRNA probes generated from non-overlapping regions of the brain. We have now characterized changes which occur as a result of the beginning of an inflammatory infiltrate in and around the vasculature. This infiltrate consisted primarily of neutrophils; however, monocytes were present. Infiltration occurred much more quickly and to a greater extent in represented tissues than in permanently occluded tissues, where it began approximately 12 hours post occlusion. Three days after surgery, in addition to the neutrophils, a heavy macrophage infiltrate was present in the reperfused tissues. Macrophages were a minor component of the permanently occluded lesions. Following five days of reperfusion, the necrotic tissue was largely replaced by macrophages, with numerous neutrophils distributed throughout the lesion. In contrast, in the permanently occluded tissues, macrophages were scattered through a loose extracellular matrix and remaining necrotic tissue, and neutrophils were located focially. The data indicate that duration of occlusion affected the size of the lesion, while reperfusion affected the timing, extent and composition of the response that leads to resolution of the necrotic tissue. Histologic and immunohistochemical evidence of these changes will be presented.
434.5 DIFFERENTIAL REGULATION OF THE HUMAN CORTICOTROPIN-RELEASING HORMONE GENE IN JEG-3 AND PC-12 CELLS. I.L.Guardoni*, B.轨道, A. Schmuck, & A. Lawrence. Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

Hypothalamic neurons mediate the mammalian stress response by integrating multiple synaptic inputs from higher brain centers and producing and secreting factors such as corticotropin-releasing hormone (CRH). Such integration at the hypothalamic level involves various second messenger systems and steroids that act by regulating transcription of the CRH gene. We are using gene transfer and DNA footprinting to localize regulatory elements in the human CRH gene. Chimeric genes containing human CRH gene 5'-flanking sequence and the chicken chloramphenicol acetyltransferase (CAT) cDNA sequence were transiently transfected into JEG-3 (human choriocarcinoma) and PC-12 (rat pheochromocytoma) cells. A CAT responsive element (CRE) has been identified for the rat CRH gene (-238 to -182) and is highly conserved in the human CRH gene. Addition of 25 μM forskolin results in a 7 fold increase in CAT activity in JEG-3 cells and 15 fold increase in CAT activity in PC-12 cells. Transfected PC-12 cells respond synergistically to forskolin and dexamethasone treatment resulting in 3 fold increase in CAT activity. In contrast, forskolin treated JEG-3 cells show a 35% inhibition of cAMP-induced CAT activity after addition of dexamethasone. DNA footprint analysis has revealed a protected region across the CRE consensus sequence in the human CRH gene. Chimeric constructs containing a mutation in the CRE result in loss of both forskolin responsiveness and protein binding. We are presently localizing the positive and negative glucocorticoid responsive elements as well as glucocorticoid receptor binding sites in the CRH gene. This will aid in understanding the molecular mechanisms involved in the "crosstalk" between cAMP and steroid pathways that result in changes in gene expression. This work was supported by a NARSAD Young Investigators Award, and NIH Grant DK42730 to A.F.S.

434.7 EFFECTS OF CHRONIC DEXAMETHASONE ON MOUSE POMC GENE TRANSCRIPTION FROM UPSTREAM START SITES. M. Wotowycz,* J. R. Lundberg,* N. Levin,*, L. Roberts. Fishberg Research Center for Neurobiology, Mt. Sinai School of Medicine, N.Y., N.Y. 10029.

Glucocorticoids inhibit transcription of the POMC gene by a proposed mechanism involving inhibition of the binding of activating transcription factors, such as fos and jun, to the POMC promoter by the glucocorticoid receptor. The POMC gene is transcribed in the pituitary to a major mRNA species of 1100 nucleotides and to minor and smaller species being detected in the pituitary as well as in many non-pituitary tissues. Recently many ACTH-secreting tumors have been demonstrated to contain cortisol-responsive gene elements, that comprise a relatively large percentage of the total POMC mRNA level, and that originate from transcription initiated upstream from the usual pituitary start site. In the present study, RNase protection assays have been used to examine the regulation of upstream POMC gene transcription in AT20 cells treated with or without glucocorticoids. mRNA expression was examined in the glucocorticoid receptor and activating transcription factors to upstream elements relative to those downstream. Since fos and jun have been shown to induce POMC gene transcription (Loeffler, Bouffier, Lorang, and Roberts, unpublished), further studies will be directed at the effects of glucocorticoids on fos gene expression in these cells.

434.9 BASAL HYPOTHALAMIC-PITUITARY-ADRENAL AXIS FUNCTION IN THE AGED RAT. Morano M.I., Vargues D.M., Casamao C.A., Kasp S.P., Watson S.J., Akl H. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The aged rat shows a delayed recovery to basal corticotropin levels following stressors. We have previously demonstrated that there is a decrease in the binding capacity of both glucocorticoid receptor (GCR) and mineralocorticoid (MR) receptors in the hippocampus of aged Fisher-344 rats, although the basal plasma corticosterone levels are similar to those of young rats. In order to further investigate the consequences of this loss of GR and MR, we have examined the basal status of the HP axis in young (6 mo) and old (27 mo) male rats. CRF mRNA content in the paraventricular hypothalamic nucleus (PVN) was quantitated by RNase protection assay. Corticosterone, ACTH, β-endorphin (βE) and Nacre (NAc) were measured in plasma, ACTH, βE or NAc EL and POMC mRNA were measured in the pituitary, anterior and posterior intermediate lobes (AL and IL). Quantitation of POMC peptide biosynthesis was determined by [3H]Leu pulse labelling and chase of AL and IL dispersed cells, followed by immunoprecipitation and SDS-PAGE. POMC mRNA content is similar in the PVN of young and old rats. In contrast, POMC mRNA levels are lower in the AL of old animals (Y = 100 ± 8% vs 0 = 59 ± 9%, p < 0.005). However, the POMC peptide levels are not changed in the AL and plasma of aged rats with respect to young ones. After 15 min pulse, de novo AL POMC synthesis of aged rats was stimulated 3 fold higher than in young rats. In contrast, POMC mRNA levels are 40% decreased in the older animals. We have also demonstrated that in AL, POMC has a shorter half-life in old rats as may account for the maintenance of a basal HP function similar to that observed in young ones. Supported in part by MH49225, DA20265 and Merck Foundation.

434.10 CRH, ACTH, AND CORTICOSTEROIDE INDUCE THE C-FOS PROTEIN IN GABAERGIC NERVE TERMINALS. S. Eriqat* and R. B. Wurtman. Department of Neurology, Stanford University, Stanford, CA 94305.

We have shown that adrenalectomy (Jacobson et al., Endocrinology 89, 1915, 1971) of rats in vivo containing CRF and stress induces c-fos mRNA in cortex, septum, hypothalamus, and other regions (Sharp et al., J. Neurosci., 1991). We have also examined the transient induction of c-fos mRNA expression in AtT-20 cells and appear to be partly mediated by protein kinase C. Stress-induced c-fos mRNA expression is activated by cAMP in a time and dose dependent manner. The stress-induced c-fos mRNA expression is activated by cAMP in a time and dose dependent manner. The feedback inhibition of c-fos mRNA expression by cAMP and the feedback inhibition of c-fos mRNA expression by cAMP is reduced by forskolin and dexamethasone treatment resulting in 3 fold increase in CAT mRNA expression of CRF in the IL during development and that this effect disappears as the IL undergoes a maturation process closely linked to the arrival of dopaminergic fibres at p3. Thus the transient induction of c-fos mRNA by CRF appears to be a critical step in CRF induced POMC mRNA expression in AtT-20 cells and appears to be partly mediated by protein kinase C.

Corticosterone (at the site of the hypothalamic-paraventricular-adrenal axis) is imperfectly understood with differences in the effects of peripheral minendocorticoid and glucocorticoid receptors. l l
tissue-specific regulator of glucocorticoid access to and activation of

CELL CULTURE. W.M. Fu and M. M. Poo. Dep. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Pathway by agents known to elevate cAMP. Goldfish bipolar cells were

ELEVATION OF CYCLIC AMP. Ruth Heidelberger and Gary Matthews. Dep. of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan and Dep. of Biological Sciences, Columbia University, New York, N.Y. 10027.

Application of a membrane-permeable form of cAMP and agents that activates adenylyl cyclase system to isolated embryonic Xenopus myocyte in culture markedly alters the ACh sensitivity of the muscle membrane, as assayed by focal extracellular iontophoresis of ACh: the ACh-response is elevated and accompanied by an increase rate of desensitization. Single channel recordings showed that the rate of adenylyl cyclase system increases opening probability and the mean open time of ACh-activated channel, without affecting conductances. These effects on ACh channels disappeared in older myocytes as well as at extrasympathetic regions of innervated myocytes. One would suggest the confinement of susceptibility to the synaptic regions following innervation. Possible involvement of cAMP second messenger system in the physiological modulation of synaptic functions was further supported by the markedly enhanced spontaneous activity at developing Xenopus neuromuscular synapses by agents that activate adenylyl cyclase. cAMP and agents co-released with ACh, if acts through adenylyl cyclase, system, could thus help the synaptic maturation by elevating the efficacy of the synapse.


Whole-cell recording techniques were used to investigate how phosphorylation modulates the rate of voltage-dependent inactivation of the voltage-activated barium current in Helix neurons. The amplitude of the barium current rapidly ran down while the rate of inactivation greatly increased if either (1) neurons were not dialyzed with ATP, or (2) neurons were dialyzed with alkaline phosphatase. ATP5 δ dialyzed partially the rate of rundown in non ATP-dialyzed neurons, and more drastically reduced the rate of both activation and inactivation of the barium current. Dialysis with the protein phosphatase inhibitor okadaic acid enhanced the amplitude and decreased the rate of inactivation of the barium current, without significantly altering the rate of rundown. Dialysis with either okadaic acid, 8-Br-cAMP, or the catalytic subunit of protein kinase A, or synthetic peptide inhibitors of protein kinases A or C did not significantly alter the amplitude or kinetics of the barium current, whereas dialysis with a synthetic peptide inhibitor of the calcium/calmodulin-dependent protein kinase significantly reduced the rate of inactivation. Therefore, a number of observations support the idea that phosphorylation of the barium current in Helix neurons is modulated by the state of phosphorylation of either the calcium channels themselves or an associated regulatory protein.
SYNERGISTIC ACTIVATION OF PROTEIN KINASE C BY ARACHIDONIC ACID TO PHYSIOLOGIC LEVELS. IN VITRO STUDIES (LESTER ET AL. SOCIETY FOR NEUROSCIENCE 1991) HAVE EVINced THAT SUBMAXIMAL ACTIVATING CONCENTRATIONS OF AA AND DAG ACT SYNERGISTICALLY TO ACTIVATE PKC. IN THE PRESENT STUDY, WE TEST THIS HYPOTHESIS OF SYNERGISTIC PKC ACTIVATION IN A CELLULAR SYSTEM. FOR THIS PURPOSE, WE USED THE HERMISSEND A NEUROSECRETORY CELL. MEASURED BOTH lA AND lc IN THE AXOTOMIZED HERRMISSEND A B CELL. BATH APPLICATION OF THE CELL PERMEANT DIACYLGLYCEROL ANALOG, 1-OLEOYL-2-ACYTETYLGlycerol (OAG 5 μM) DID NOT HAVE SIGNIFICANT EFFECTS ON THE TWO K+ CURRENTS AFTER 15 MIN. INCUBATION. AT 30 MIN. OAG, A 20% INCREASE IN lc AMPLITUDE WAS OBSERVED. lA REMAINED VIRTUALLY UNCHANGED. STAURUSPORINE HAD NO EFFECT ON THE lA ENHANCEMENT, SUGGESTING THAT THIS OAG EFFECT MIGHT NOT BE PKC-MEDIATED. APPLICATION OF 5 μM AA HAD NO EFFECT ON BOTH lA AND lc. APPLICATION OF 5 μM OAG 15 MIN. LATER, CAUSED A STRONG REDUCTION OF lA CONDUCTANCES. A 57% AMPLITUDE REDUCTION WAS OBSERVED FOR lA AND lc AMPLITUDE DECREASED IN 44%. STAURUSPORINE BLOCKED THESE EFFECTS. SUGGESTING THAT THESE OAG-AA EFFECTS ARE PKC-MEDIATED. PKC REGULATION OF THESE SAME K+ CURRENTS HAS BEEN SHOWN TO INDUCE RESPONSES SIMILAR TO THOSE FOUND FOR CLASSICAL CONDITIONING. THIS OAG-AA SYNERGISTIC EFFECT MAY, THEREFORE, BE AN IMPORTANT STEP IN THIS PROCESS.

INHIBITION BY LHRRH OF N-TYPE CA CURRENT IN BULLFROG SYMPATHETIC NEURONS: KINETICS AND STATE-DEPENDENCE. J. M. Roland and B. P. Brann. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Inhibition by luteinizing hormone releasing hormone (LHRRH) of Ca channel current was studied using whole-cell recordings from freshly dissociated bullfrog sympathetic ganglion neurons. LHRRH acts through activation of G-proteins to inhibit N-type but not L-type channels. The rate of development of inhibition is faster at higher [LHRRH], reaching 2 sec at saturating [LHRRH] of 0.3-3 μM. Recovery proceeds with t-19 sec and is concentration-dependent. LHRRH inhibition is strongly affected by the gating state of the channel: LHRRH has little effect if applied during a long activating voltage pulse but strongly inhibits if applied when channels are mostly in the resting state. Inhibition can be partly overcome if channels are activated by short, large depolarizations or long, smaller depolarizations. The state-dependent properties are predicted by a model that assumes: 1) Inhibition results from activated G-proteins binding to the channel, 2) G-protein binding stabilizes closed gating states, and 3) activation of G-protein bound channels destabilizes G-protein binding.

CANNABINOIDS INHIBIT A HIGH-THRESHOLD CALCIUM CURRENT. K. Meekin and B. Hills, Physiol. and Biophysics, Univ of WA, Seattle, WA 98195.

Medicinal properties of Cannabis sativa and its major biologically active constituent, Δ9-tetrahydrocannabinol (THC), have been known for years. Recently, a high-affinity THC receptor has been identified that has a CNS distribution consistent with the behavioral effects of THC. The cellular mechanisms of action of THC are uncertain. One action of THC is to inhibit adenylyl cyclase (AC). As neurotransmitters that inhibit AC often affect ion channels by other pathways, we have investigated a link between cannabinoid receptors and calcium channels. In NG108-15 cells, the cannabinomimetic alkylamidole, WIN 55,212-2, potently, stereospecifically and reversibly inhibited a component of the high-threshold voltage-sensitive ICa. Maximal inhibition of ICa was 40% at 100 nM WIN 55,212-2. A similar decrease was found with the non-classical cannabinoid, CP 55,940 (100 nM). The inhibition is not likely to be due to a decrease in cAMP level as prior incubation with 8-Bromo-cyclic AMP (100 μM), dibutyryl cAMP (1 mM) and/or isobutylmethylxanthine (100 μM) did not prevent ICa inhibition by WIN 55,212-2. The components of ICa inhibited by cannabinoids are not entirely the same as the ICa inhibited by neuropeptide Y (NPY) and adenosine (ADO) in these cells. WIN 55,212-2 inhibited a subset of the ICa inhibited by NPY (1 μM) with a slower onset of inhibition. On the other hand, WIN 55,212-2 and ADO (50 μM) inhibited distinct ICa subtypes, however the onset of inhibition was similar for the two compounds.

Given the central role of calcium channels in neurotransmitter release and in patterned firing of action potentials, it is possible that inhibition of these channels contributes to the profound behavioral changes produced by THC. Supported in part by GM07604, NS08174, and by a Research Award from the McKnight Foundation.

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435.11 REGULATION OF Kv1 CHANNEL GENE EXPRESSION IN CLONAL AND NATIVE PITUITARY CELLS. E.S. Leytan, J. Meiner*, C. Litwin, and B. Amat, Dept. of Pharmacology and Medicine, Univ. of Pittsburgh, Pittsburgh, PA 15261.

We have been exploring the hypothesis that transmitters and hormones interact with long-term changes in cell excitability and secretion by regulating ion channel gene expression. Previously, we reported that dexamethasone (DEX), a glucocorticoid receptor (GR) agonist, increases voltage-gated K+ current and mRNA encoding the Kv1 K+ channel subunit in GH3 rat pituitary tumor cells. We have now investigated the role of GR activation, and have identified a novel neuropeptide that may have an opposing effect. Kv1 RNA is increased with corticosterone (COR) and hydrocortisone (HC). This induction is inhibited by the GR receptor antagonist RU38466 and is not seen with estradiol or testosterone. To explore whether this gene regulation is physiological, native rat pituitary cells were dissociated, cultured for 3 days, and then treated with steroids. 6 hour treatments with DEX or COR increased Kv1 mRNA. ~3-fold. This induction was blocked by RU38466 and was not mimicked by estradiol. Therefore, GR activation increases K+ channel gene expression in clonal and native pituitary cells. In contrast to the glucocorticoids, the neuropeptide TRH, which increases secretion, decreased Kv1 mRNA in GH3 cells in a preliminary experiment. This suppression was also seen with BATX 8644, a K+ channel antagonist, suggesting that cytchalamic calcium may inhibit expression of Kv1. Thus, glucocorticoids and TRH may have opposing effects on secretion and K+ channel gene expression.

435.12 DEVELOPMENT OF TWO TRANSIENT POTASSIUM CURRENTS IN CULTURED MOUSE HIPPOCAMPAL NEURONS. B.L. Wies* and N.E. Barish, Division of Neurosciences, Beckman Research Institute, T. J. Hoag Hospital, T. J. Hoag Hospital, 30000 South Coast Hwy., Laguna Beach, CA 92651. We studied the potassium currents present in mouse hippocampal pyramidal neurons in dissociated cell cultures derived from embryonic day 15 embryos. Potassium currents were isolated using whole-cell patchclamp recording and were regulated by K+ channel blockers and modulators. Using a K+ channel agonist, a transient A-current was identified that was transiently increased in magnitude during the first postnatal week. Subsequently, a second transient A-current was identified that was transiently increased in magnitude during the second postnatal week. These currents were blocked by 4-aminopyridine (4-AP) and were not expressed by neurons from K+ channel mutant mice. The transient A-currents were also regulated by intracellular calcium and by extracellular potassium. The transient A-currents were expressed by neurons that were differentiated in vitro in the presence of a variety of growth factors and had the morphology of hippocampal pyramidal neurons. These results suggest that the transient A-currents are a novel type of potassium current that is expressed transiently during the early postnatal period in hippocampal neurons and may play a role in the development of the hippocampus.

436.1 VIBRATORY STIMULATION (VS) AND RECTAL PROBE ELECTROSTIMULATION (RPE) OF SPINAL CORD INJURED (SCI) MEN. N.L. Bracken and C.M. Lynne*. The Miami Project to Cure Paralysis, University of Miami, Miami, FL 33136.

Men with spinal cord injuries are often impotent and infertile due to erectile and ejaculatory dysfunction. The present study compared the quality of erections, ejaculations and semen using the methods of VS or RPE. These measures were correlated to the subjects' neurological level and completeness of injury as well as other variables. Thirteen SCI men were studied a total of 35 times. Five subjects could ejaculate with either method, five with only RPE, one with only VS and two with neither method. The ability to ejaculate correlated more with completeness of injury than with level of injury. Men who ejaculated, both antegrade and retrograde samples were recovered in most cases. Antegrade ejaculations produced by VS tended to be forcefully expelled compared to those produced RPE which lacked most motor components. Occurrence of erections prior to stimulation did not predict occurrence or quality of erections during stimulation. Subjects reported pleasurable feelings associated with each stimulation method. The normative data collected in this study are intended as a basis upon which to design future studies investigating the causes and treatments of male impotence and infertility following SCI.


Previous experiments in our laboratory have described the method used to measure the conduction velocity distribution of a selected group of fibers (Br. Res.520:83-89[1990]). Both VS and RPE were applied this technique to the diabetic rat. Ten female Sprague-Dawley animals were made diabetic with streptozotocin (60 mg/Kg iv). Six matched animals were used as controls. Two diabetics died shortly after their injections but the remaining animals survived to the completion of the experiment (approximately two months). Glycated hemoglobin values measured at the time of death were 17.19 +/- 4.74 % (diabetic, n = 8) and 4.07 +/- 0.74 % (controls, n = 6). Diabetic bladders were thicker and heavier. The wet weights were 0.50 +/- 0.11 gm (diabetic, n = 7) and 0.16 +/- 0.01 gm (controls, n = 6). The conduction velocities of a total of 151 and 86 single afferent fibers were measured in the diabetic and control animals respectively. The conduction velocity distribution of the diabetic showed a shift towards slower speeds when compared to controls. The mean conduction velocities were 1.70 m/s for diabetics and 2.84 m/s for controls. The percent of units with conduction velocities greater than 2.5 m/s was 11.3 for diabetics and 27.0 for controls. This experiment demonstrates, for the first time, that diabetes causes a significant reduction of apparent conduction velocities in a functionally well-defined system.

436.3 GALANIN IMMUNOREACTIVITY IS SEXUALLY DIMORPHIC IN AUTONOMIC REGIONS OF THE RAT LUMBOSacRAL SPINAL CORD. R.W. Newton, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72203.

Galanin-immunoreactive (GAL-LI) fibers have been demonstrated to surround preganglionic autonomic neurons; and a large group of GAL-LI neurons, which project to the thalamus, is found in lumbar laminae VII and X (Ju et al., Neuroscience, 20:439, 1987). Additional characteristic of the distribution of GAL-LI in the rat spinal cord are sexually dimorphic morphology in the lumbarosacral autonomic spinal cord. Ten male and ten female adult Sprague-Dawley rats were perfused for the PAP technique in order to reveal spinal sympathetic neurons. GAL-LI, diluted 1:1250, was used (Belmont, CA #RAS-713-N). GAL-LI was not sexually dimorphic in the thoracic spinal cord. However, GAL-LI fibers in autonomic regions of the lumbar spinal cord were more abundant in males than females. The females showed a marked variation in the number of GAL-LI fibers: this variation was subjectively classified as either "average" or "low". This variation is probably due to fluctuations in estrogen levels during the estrous cycle. However, the number of GAL-LI fibers in any female never exceeded the number found in males. The number of previously described lumbar GAL-LI neurons was also sexually dimorphic: males have significantly (P < 0.01) more GAL-LI neurons (62%) than females. The number of GAL-LI neurons in females was not variable. The physiological significance of sexually dimorphic, autonomic, GAL-LI is presently unknown. Supported by BRSG KR05350 and the Arkansas Caduceus Club.

436.4 IN VITRO NEONATAL RAT: INTRACELLULAR RESPONSES OF BRAINSTEM NEURONS TO SUBDIAPHRAGMATIC VAGAL STIMULATION. C.S. Yuan* and W.D. Barber. Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724.

We developed an in vitro neonatal rat preparation to investigate brainstem neuronal processing of subdiaphragmatic vagal afferent fibers. Previous experiments in our laboratory have described the neuronal processing of subdiaphragmatic vagal input. (Barber et al., FASEB J. 5(5): A1063, 1991). Neonatal rats, 0 to 2 days of age, were anesthetized with halothane. Following a craniotomy, the brainstem was transected at the rostral medulla and the stomach with intact vagi and caudal brainstem was isolated. The preparation was superfused in a Krebs solution. We developed an in vitro rat brainstem preparation for subdiaphragmatic vagus nerve stimulation. The subdiaphragmatic vagus nerve was stimulated in a maximal stimulation solution. We used intracellular recordings to investigate brainstem neuronal responses to subdiaphragmatic vagal input without the effects of general anesthesia. (Supported by USPHS Grants NS 27972 and DK 36289).
436.5
ORGANIZATION OF MEDULLARY AUTONOMIC REFLEX ARCS.
ORGANIZATION OF MEDULLARY AUTONOMIC REFLEX ARCS.

Medullary autonomic reflex arcs are composed of 1° visceral afferents ending
in nucleus tractus solitarii (NTS), relaying by direct and indirect
pathways to autonomic preganglionic and respiratory lower motor neurons.
Polysynaptic limbs of reflex arcs are thought to involve interneurons in the
lateral tegmental field (LTF). We sought to define interneuronal networks in the
medullary LTF and their relationships to the solitario-reticulospinal
(transsegmental) tract bridging NTS and autonomic premotor pools in the
ventrolateral medulla (VLM). In adult male anesthetized (0.5 mg/kg
chloral hydrate, i.p.) rats, injections of two retrograde fluorescent tracers (rhodamine
microbes and FluoroGold) were centered on NTS and the rostral
ventrolateral reticular nucleus (RLV); distributions of single and double-
labelled neurons were mapped in the medulla. NTS and RLV afferent
projection neurons were backfilled along elongate sheets concentrated in the
intermediate reticular zone of LTF; most formed independent populations.

Neurons colocalizing both tracers were detected in a dorsal reticulotegmental
(LTFd) adjacent to NTS. PHA-L injections into LTFd confirmed divergent
projections to NTS and VLM. Methods combining anterograde transport of
PHA-L with retrograde tracers were developed to determine relationships
between the solitario-reticular tract (SRT) and RLV afferents. RLV-
fluorescent interneurons were distributed throughout LTF; and embedded
within immunofluorescent fibers of SRT. An interstitial nucleus of the SRT
extending from the NTS to VLM may be an anatomical substrate of
multisynaptic autonomic reflex arcs.

436.6
EFFECT OF SOMATOSTATIN-28 (SS) ON VAGAL CARDIOMOTOR NEURON
(VCN) FUNCTION. M. Cameron, C.W. Miller* and E.T. Kirikopeoulus,
Dept. of Anatomy & Physiology, Univ. of Western Ont., London, Canada.

We have shown that SS-immunoreactive fibers are located around VCN in
nucleus ambiguus (AMB). The present studies investigated the source of SS
afferents to AMB and the effect of SS on VCN function. Experiments were done in
adult male Sprague-Dawley rats. In the first series, rats were anesthetized
with pentobarbital, the femoral artery was cannulated and heart rate (HR) was
monitored. Double-barreled glass micropipettes were made. Cardiomyosin regions
were identified by the vagal bradycardia evoked by L-glutamate
microinjections (10-30 nl/mL), and these sites received injections of the
retrograde tracer Fluorogold (2%/10 nl). After 7-10 days animals were
colicollimated, perfused 8-10 hr later, and the forebrain and brain stem were
processed using immunofluorescence to identify neurons containing SS and FG.
Double-labeled neurons were found in the central amygdaloid nucleus, medial
paraventricular divisions of the hypothalamic paraventricular nucleus and the
central nucleus of the NTS. In the second study, animals (n=32) were
anesthetized with alpha-chloralose, artificially ventilated and spinal cord
transected at C4-C5. The femoral artery and vein were cannulated and arterial
pressure (AP) and HR were monitored. Microinjections of L-glutamate were made to
identify cardiomyosin regions of AMB and these sites subsequently received
injections of SS (50-100 nl; 5-10mmol). SS consistently elicited a slow
onset (6-33min), long-duration (10-30min) increase in HR that was not
accompanied by a change in AP. The HR response to SS was ±22±12 bpm
(5-60 bpm) from a control HR of 256±30 bpm. Control injections of 0.9% saline
(n=6) did not alter HR. These data suggest that SS inhibits VCN function and that limial,
hypothalamic and/or medullary SS-containing cell groups projecting directly
to cardiomyosin regions of AMB may provide the anatomical
pathways by which these SS effects are mediated. (Supported by the MRC.)

436.7
EXCITATORY EFFECTS OF THYROTROPHIN-RELEASING HORMONE
(TRH) IN DORSAL MOTOR NUCLEUS OF THE VAGUS (DMV)
MOTORUNNEURONS. A.A. Trevel and R.A. Giuli. Y.M. Hernandez, and
S.Giacometti, Dept. of Anatomical Neurosciences, U Mass., W. Med.,
DC 20007

The purpose of our studies was to characterize the excitatory effect of TRH
in DMV motor neurons by using the patch-clamp technique in
vtaior brain slices. In our initial studies we employed the cell-attached configuration
using concentrations of TRH ranging from 3 to 30 pM. Exposure of DMV
motor neurons to TRH resulted in dose-related increases in spontaneous
activity potential firing rate (2-4-fold increase at the maximal
TRH concentration tested). This was observed in all the DMV neurons tested
(N=21) independently of their location rostral or caudal to the obex.

In two series of experiments we used the whole-cell configuration
and TRH was applied directly to the cell from the patch pipette. At
saturation (2000 ng/L, 1000 ng/L) TRH increased frequency of
spontaneously occurring excitatory postsynaptic
postsynaptic currents and, in some cases, a decrease in spontaneous inhibitory
developed throughout LTF; and embedded
within immunofluorescent fibers of SRT. An interstitial nucleus of the SRT
extending from the NTS to VLM may be an anatomical substrate of
multisynaptic autonomic reflex arcs.

436.8
EXPRESSION OF C-FOS IN NEURONS OF THE BRAINSTEM
DURING HYPOXEMIA IN THE SHEEP. G.E. Hoffman, T.J.
McDonald*, A. Sved, and P.W. Nathanielsz. Depts. Physiol. and
Behav. Neurosci., Univ. Pittsburgh, Sch. Med., Pittsburgh, PA 15261 and Lab. for

The fetal pituitary adrenal axis is responsive to hypoxemia; fetal ACTH levels rise and c-fos expression in corticotropin- 
releasing hormone (CRH) neurons as well as other neurons in the
paraventricular nucleus (PVN) of the hypothalamus is induced.
Within the brainstem, the ventrolateral medulla and nucleus of the
solitary tract (NTS), are sensitive to hypoxia and can influence the
hypothalamus. Using c-fos as a marker for neuronal stimulation
we examined the relationship between activation of these
brainstem centers (including corticotropin releasing neurons within them)
and the PVN. Fetuses (123±12 days gestational age) were made
hypoxic by reducing the maternal oxygen to 60% of normal for
20-60 min and the brains were perfused and immunocytochemically
stained for c-fos and CRH (hypothalamus) or DBH (brainstem).
Following hypoxemia, the induction of c-fos in CRH and
other PVN neurons was accompanied by a marked
activation of c-fos in the ventrolateral medulla and NTS;
activation included catecholamine neurons in the A1/C1 and A2
C2 cell groups. The degree of c-fos activation of brainstem
cells was correlated with the magnitude of PVN activation.

These data support the hypothesis that fetal CRH neurons are
stimulated during fetal stress, and suggest that catecholamine systems might mediate that stimulation.

Supported by NIH HD 21350 and NS 38477, and NS 23858.

436.9
PARAVENTRICULAR NUCLEUS (PVN) OXYTOCIN NEURONS ARE
RESISTANT TO THE EXCITATORY NEUROTOXIN, IBOTENIC ACID.
of Physiology and Pharmacology, Wake Forest University Medical Center, Winston
Salem, NC, 27103.

Oxytocin-like responses of parvocellular PVN neurons attenuate the tachycardia and plasma oxytocin (OXY) responses to
stress. We examined the effects of this lesion on brain OXY mRNA and peptide levels in plasma, posterior pituitary (PP),
brainstem (BS), olfactory tubercule (OLT) and spinal cord. Male rats (10 days post oper) were given water or 2% salt to drink for 72
hrs. Rats received injections of SS (20-100 nl;5-10mmol). SS consistently elicited a slow
onset (6-33min), long-duration (10-30min) increase in HR that was not
accompanied by a change in AP. The HR response to SS was ±22±12 bpm
(5-60 bpm) from a control HR of 256±30 bpm. Control injections of 0.9% saline
(n=6) did not alter HR. These data suggest that SS inhibits VCN function and that limial,
hypothalamic and/or medullary SS-containing cell groups projecting directly
to cardiomyosin regions of AMB may provide the anatomical
pathways by which these SS effects are mediated. (Supported by the MRC.)

Supported by NIH HD 21350 and NS 38477, and NS 23858.

436.10
BARORECEPTOR NERVES INFLUENCE HYPOTHALAMIC PEPTIDE mRNA
Department of Physiology and Pharmacology, Bowman Gray School of Medicine
of Wake Forest University. Winston-Salem, NC 27103

Neuroendocrinological and physiological evidence indicates that baroreceptors influence hypothalamic vasopressin (VP)
and oxytocin (OT) expression. We have conducted an effect of sinoaortic denervation (SAD) on the expression
of VP and OT mRNA under basal and stimulated conditions. Male control or SAD
rats (10 days post oper) were given saline or 2% salt to drink for 72 hrs. Rats received
bilateral VP injections of SS (20-100 ng;5-10mmol) and were sacrificed at 7
days. The brains were fixed and processed for in situ hybridization using an
oligonucleotide probe. Oxytocin-like responses of parvocellular PVN neurons were
observed in SAD animals. There were no changes in central OT content with
salt loading produced
an increase in PVN and SON VP content in control animals (1.0+0.2 vs 2.8+0.5
ng/PVN and 1.6+0.2 vs 3.2+0.4 ng/SON; water vs salt loading). No changes
there was increased expression under basal and stimulated conditions in the
denervated animals. This suggests that denervation activates the hypothalamic
synthesis. (Supported by HL 43178.)

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THURSDAY AM REGULATION OF AUTONOMIC FUNCTIONS 1099

The R15 polyprotein gene in a marine mollusk Aplysia encodes a primary mRNA transcript, alternatively spliced in different neurons to generate overlapping sets of neuropeptides (Buck et al., Cell 51:127, 1987), including R15α, (38 a.a.) and R15α2, (24 a.a.), which exert coordinated actions on invertebrate reproductive, digestive and reproductive systems. We sought to determine whether R15α, and R15α2, are expressed in rodent brain and adrenal gland. Adult male anesthetized rats were perfused; tissues incubated in rabbit polyclonal R15α, (16 mer difference) or R15α2, antisera and immunoprocessed. R15α1-lir was restricted to locus ceruleus, epinephal and adrenal chromaffin cells; whereas R15α2, was expressed by neurons in nucleus tractus solitarii, paraventricular and supraoptic (SO) hypothalamic neurosecretory nuclei, and processes in neurohypophysis. To determine whether R15α2, is coexpressed in vasopressinergic tissues, we sequentially incubated in R15α2, antisemur and arginine vasopressin (AVP) antisemur (24h; immunofluorescence). Neurons expressing R15α2, and AVP were topographically segregated. Distributions of R15α2, and AVP neurons in paraventricular divisions of PVN were skewed anteroverventromedially to the AVP population. In the supraoptic nucleus R15α2, neurons formed a shell encircling an AVP-lir cell column. Striking parallels exist in localization of bioactive invertebrate neuropeptides to autonomic and neuroendocrine control sites in the mammal.


The aim of this study was to investigate the role of the brain noradrenergic systems in modulating brain DNA synthesis (BDS) during nonassociative learning (Soc Neurosci Abstr., 15, 916, 1990). Adult male Sprague-Dawley rats were randomly assigned to one of six groups. Group A and B were bilaterally lesioned in the dorsal noradrenal bundle (CNB) by injection of 6-OH-DA, group C and D were sham-lesioned, and group E and F were unoperated unlesioned animals. Rats were given 50μl H-thymidine intraventricularly and sacrificed 0.5h later. The exposed rats were injected 15 min before test trial, Groups B, D and F were exposed for 10min to a L-at-maze, and corner-crossings and reentries were monitored per 1min-blocks. BDS was measured in several brain areas. H-thymidine into DNA. DNB-lesioned rats exposed to the L-at-maze did not show the marked inhibition in BDS observed under comparable conditions in control rats. The results support a role of noradrenergic systems in modulating brain DNA synthesis during learning. (Supported by CNR and MURST 40% grants).


The relationship between the magnitude of the error in the context of temporal memory and sodium-dependent high affinity choline uptake (SDHACU) in the hippocampus and frontal cortex was examined in mature (10-16 mo) and aged (24-30 mo) rats. The peak time of the response rate distribution that relates the probability of a response to signal duration in a 20-sec peak-interval (PI) timing procedure was used to index the remembered time of reinforcement. Discrepancies in temporal memory produce stable horizontal displacements of timing. Memory facilitation by aFGF was significantly expressed in both groups of rats, with a peak time of the temporal memory and sodium-dependent high affinity choline uptake (HACU) being very close to actual time of reinforcement, with a symmetrical distribution of individual peak times centered around that time. In contrast, as rats age they demonstrate a proportional rightward shift in their timing functions, indicating that remembered durations overestimate the actual time of reinforcement by a constant percentage. Regression analysis of the data from chronic-run rats, however, showed that the correlation coefficients averaged over both age groups were r = .75, p < .001 and r = .06, p > .05 for the frontal cortex and hippocampus, respectively.

The behavioral evidence supporting the hypothesis that the Naples High (NHE) and Low-excitability (NLE) rats may be a genetic model to study hippocampal functions (Adv. Biosci., 59:299, 1986) is presented. It comprises: (1) Differential activity in a spatial novelty situation (selective trait), proportional to the stimulus complexity rats are exposed to; NHE are hyper- and NLE- rats hypactive; (2) Unimpaired acquisition rate of water-motivated operated conditioned in both lines vs. controls; and (3) Impaired working memory in a non-reinforced tunnel maze with 6 asymmetrical arms in both lines compared to random-bred controls (Adv. Biosci., 70:177, 1988). Thus, a defective hippocampal-pendently spatial processing is inferred. The behavioral evidence (qualitatively similar in both lines), along with histochemical (Behav. Brain Res., 24, 111, 1987), electrophysiological and neurochemical one (Adv. Biosci., 59:299, 1986; Behav. Brain Res., 32, 287, 1989), suggests that both NHE/NLE are disintegrated at the hippocampal interface. Moreover, far from fitting in current theories on hippocampal functions, they lend support to its modulatory role in the parallel processing of cognitive and non-cognitive (attentional, emotional) informational components. (Supported by CNR and MURST 40% grants).

437.6 COGNITIVE AND NEUROCHEMICAL CHANGES INDUCED BY EXPERIMENTAL AUDITORY DEFICIT IN AGED RATS. J. Drogo, L. Nardone and F. Spadaro. Department of Pharmacology, University of Catania Medical School, Catania, Italy.

In aging, auditory deficits occur frequently and have been involved in cognitive impairment. We studied the effects of an experimental acoustic deafferentation on learning and memory processes and on some neurochemical parameters of aged rats. Sprague-Dawley male rats, 18 months old, were used. A solution of KCl (10% v/v) and Fentanyl was injected once a week for three months in the middle aged male rats. Learning and memory capacity, assessed with passive and active avoidance tasks, was reduced in rats with experimental auditory deficit as compared to control animals. In the despair test, a behavioral model for depression, animals with the auditory impairment, showed a more serious depressive attitude than controls. After the behavioral tests were performed, an auditory recovery of a group of animals was allowed suspending the injections. A month later, the behavioral testing was repeated. The animals that recovered from the auditory deficit showed a normalization of the behavioral pattern. A neurochemical study showed a reduction in 5A and DOPAC content and in CAT and AChE activity in various brain areas of animals with auditory deficit. These results show that prolonged acoustic deafferentation can induce reversible cognitive changes. This may be of clinical relevance in elderly people with an acoustic deficit without hearing aids.


Nimodipine, a dihydropyridine calcium channel antagonist, facilitates learning in aging rabbits (Deyo et al., 1989) and increases spontaneous firing rates of CA1 pyramidal neurons in vivo (Thompson et al., 1990). CA1 neurons from trace conditioned rabbits also show increased trace-specific dendritic afterhyperpolarizations (AHP) reductions (dchenge et al., 1990). To investigate the mechanism by which nimodipine increases excitability, we used the AHP before and after application of nimodipine in both aging and young adult CA1 neurons in vitro. Intracellular recordings were made from 151 CA1 neurons (87 young, 64 aging).

Doses 100 mM nimodipine had no effects on young CA1. Aging CA1 neurons showed significantly reduced AHP amplitudes and areas, and decreased accommodation after 100 mM nimodipine (extracellular levels in behavioral studies). Aging CA1 neurons also showed significantly larger AHPs than young CA1 neurons. These data support the hypothesis that aging neurons have altered calcium regulation (perhaps via decreased calcium buffering capacities) resulting in larger AHPs, and that in aging CA1 neurons, nimodipine can decrease the AHP, thus increasing cellular excitability in a manner facilitating learning. (Supported by I K01 AG08796 & The Mlin Institute).


Previous work on the role of the primate hippocampus in spatial processing has shown that there are cells in the macaque hippocampus which respond when the monkey looks at particular locations in space, and that some of these cells respond in allocentric (world-based) rather than egocentric (body-centered) coordinates (J.D. Peigenbaum & E.T. Rolls, 1991, Psychobiology 19: 21-40). Such cells were named ‘space’ cells, to distinguish them from ‘place’ cells in the rat which respond when the rat is in a particular place in an environment. In the experiments described here, we investigated activity changes of cells when macaques (Macaca fascicularis) were moved in a small chair on wheels to different places in a cue-controlled testing environment (a 2 x 2 chamber). Many cells with spatial responses were recorded in the primate hippocampus and parahippocampal gyrus. For a number of cells, it was possible to show that firing rate depended on where in space the monkey looked, and not on the place in the environment, or on head direction. The responses of some cells were affected by the removal of cues in the environment. Other cells responded in relation to whole body movement of the monkey in the testing environment, with some specific for head-centred rotation, and others for linear translation. Cells which responded according to the place where the monkey is located and independently of local view and of head direction have not yet been found, but testing is continuing.

437.9 SINGLE NEURONS IN PRIMATE TEMPORAL CORTEX CODE BOTH OF PAIRED ASSOCIATES STORED IN VISUAL LONG-TERM MEMORY. K. Sakai & Y. Miyashita. Dept. of Physiology, Sch. of Medicine, Univ. of Tokyo, Tokyo 113, JAPAN.

Human memory is assessed by the paired associate learning test, in which the subject is shown a series of paired figures and then asked to retrieve the associate of a cue figure. We prepared 24 computer-generated patterns during the cue period, and sorted geometrically distinct figures into pairs. Two monkeys (Macaca fuscata) were trained to memorize these pairs. In each trial, a cue stimulus was presented on a video monitor, and choice stimuli were shown after a delay period.

Each monkey obtained a fruit-juice reward following correctly touching the paired associate. We found two types of neurons in the anteromedial temporal cortex in rhesus monkeys. One type selectively responded to both of the paired patterns during the cue period. The other type, which responded to one pattern optimally, exhibited tonic activity during the delay period after that pattern was used as a cue. These results provide direct evidence that the single neurons acquired the response selectivity through associative learning, representing long-term memory of paired associates.


The hippocampus has long been considered important in the consolidation of memory. In 1989, a "tracing circuit" model of human memory was proposed when "impulse bursts occurring during the consolidation of memory by a trace circuit" were observed. The existence of reciprocal cortico-hippocampal projections suggests that the hippocampus can be formed and can further consolidate the tracing circuits in the cortex. Here, using recent compelling data on a longitudinal (ipsilateral) and cross-projection of the hippocampus, I propose a "tracing circuits association" model, in which association of episodic memories of different modalities can form a new closed circuit through the hippocampus by reciprocal projections with the association cortex, the tracing circuits are further stabilized, with prolongation of firing in closed circuit. CA3 neurons appear to project to CA1 neuron and distributed along the longitudinal axis of the hippocampus (Ishizuka et al., J.Comp. Neurol. 295, 580,1990), and by different closed circuits of different modalities can easily be connected by cross projection of CA3 neurons in one cortico-hippocampal circuit and CA1 neurons in a different circuit. Such crossed circuit CA3-CA1 synapses are repetitively stimulated during the prolonged firing, induced LTD and other facilitatory processes, which stabilize these synaptic connections over long periods, enabling reactivation of the associative memory circuit.
437.11

A SPIKING NEURON NETWORK MODEL OF DYNAMIC SHORT-TERM MEMORY. B. Kehoe* and D. Zipser. Department of Physics, CSUF, Fullerton, CA 92834 and Department of Cognitive Science, UCSD, La Jolla, CA 92039.

The firing patterns of cortical neurons in monkeys engaged in short-term memory tasks (e.g., Fuster, J.M., J. of Neuropsychology, 36, 61-78, 1973) have been shown to be similar to the activity patterns of units in a recurrently connected, constantly updated, artificial neural network (ANN) trained as a gated sample and hold register (D. Zipser, Neural Computation, 3, 178-192, 1991). This similarity suggests a dynamic storage mechanism for short-term memory utilizing a recurrent network. The discrete time model has been extended to an approximation of a continuous time ANN and also to a model of a single neuron, based upon the continuous model, trained using the backpropagation through time algorithm subject to biologically based constraints. The characteristic features of the discrete network corresponding to the neurobiological data are preserved. Layer networks have been trained with significant levels of noise to reflect the variability of average spiking rate of real neurons. The resulting trained network spontaneously learns to utilize all of its resources by creating clusters of similarly connected units that represent what were single units in smaller networks. These clusters presumably help to overcome the effects of noise by averaging. Networks made up of spiking units, with biologically based parameters and connections determined by the continuous network, are being studied. Their interspike interval distributions model those found in the data. The attractor dynamics of spiking networks, and the implications of those dynamics for perturbed neurons in such a network, are explored as a guide to further experimental testing of the recurrent model of dynamic short-term memory.

437.13

CORTICAL ASSOCIATIVE MEMORY FUNCTION AND ACETYLCHOLINE: A COMPUTATIONAL MODEL.

M.E. Hausalimo, Dept. of Psychology, Harvard University, Cambridge, MA 02138.

Psychopharmacological evidence suggests a role for acetylcholine in memory function. Computational modeling of cortical associative memory function may help link this role in memory to the specific effects of acetylcholine on cortical neurons. Acetylcholine (100μM, presented with 1μM neostigmine) selectively suppresses intrinsic fiber synaptic transmission in slice preparations of piriform cortex, while leaving afferent fiber synaptic transmission unaffected (Hausalimo and Bower, J. Neurophysiol., in press). A model of piriform cortex shows that this selective suppression of excitatory feedback, applied during learning of new input patterns, can greatly enhance associative memory performance. Without this suppression, excitatory feedback generates previously stored patterns during the learning of new patterns which activate any of the same neurons. This leads to the strengthening of connections between all neurons activated by overlapping input patterns, resulting in considerable interference between stored patterns. Cholinergic suppression of excitatory feedback can prevent this problem by decreasing the activity of neurons not receiving direct afferent input during learning, thereby satisfying the following condition:

\[ \mu > \sum_{i=1}^{n} \left( c_i \cdot \psi_i(g_i - g_{\text{output}}) \right) \]

where \( \mu \) = synaptic modification threshold, \( \Omega \) = output function threshold, \( c = \) neuron activation, \( c = \) cholinergic suppression, \( B = \) excitatory feedback strength, \( H_i = \) inhibitory feedback function. This has an effect similar to clamping neuron activity to the input pattern during learning, a technique used in the modeling literature, but not previously justified by neurophysiological data. Memory deficits associated with presentation of cholinergic antagonists show some characteristics of the interference predicted by this model. This work suggests how the memory deficits associated with Alzheimer's disease could arise from a loss of cortical cholinergic innervation. (Support: NIH grant NS07251, and The French Foundation for Alzheimer Research.)

CHEMICAL SENSORS: PERIPHERAL MECHANISMS III

438.1

ROLE OF CAMP PHOSPHODIESTERASE IN OLFACTORY SIGNAL TRANSDUCTION. F.F. Bolton-Cloud, G.V. Ronnett* and A. Cunningham. Hopkins University School of Medicine, Baltimore, MD 21205.

Pharmacological evidence suggests a role for cAMP in olfactory signal transduction. At least two forms of PDE, CA2P and CA3P, are present in olfactory tissues as determined by biochemical and immunohistochemical methods. High affinity CA2P PDE appears to be the predominant form in whole cell preparations of olfactory tissue and is fivefold greater in brain than assayed at low micromolar CAMP. By comparison, in turbinates, both the PDE activity is sensitive to isopropryl. Following blockage with CA3P PDE, the CAMP PDE activity is significantly depleted in turbinates. Immunocytological staining of the rat olfactory mucosa reveals selective expression of CA3P in olfactory receptor neurons with immunocytochemically active PDE (IPS-PDE) in the dendritic knobs and cilia, as well as the axon bundles. Nonspecific cells or basal cells display any immunoreactivity. Blockage greatly depletes the population of IPS-PDE positive neurons. This gradient in CAMP PDE activity is significant with Mfn concentrations of CAMP outside. Application of protein kinase A activator increases the response of ORNs to odorants, showing that CA3P PDE channel to open in response to odorant stimulation. This is significant with Mfn concentrations of CAMP outside. Application of protein kinase A activator increases the response of ORNs to odorants, showing that CA3P PDE channel to open in response to odorant stimulation.

438.2


The firing patterns of cortical neurons in monkeys engaged in short-term memory tasks (e.g., Fuster, J.M., J. of Neuropsychology, 36, 61-78, 1973) have been shown to be similar to the activity patterns of units in a recurrently connected, constantly updated, artificial neural network (ANN) trained as a gated sample and hold register (D. Zipser, Neural Computation, 3, 178-192, 1991). This similarity suggests a dynamic storage mechanism for short-term memory utilizing a recurrent network. The discrete time model has been extended to an approximation of a continuous time ANN and also to a model of a single neuron, based upon the continuous model, trained using the backpropagation through time algorithm subject to biologically based constraints. The characteristic features of the discrete network corresponding to the neurobiological data are preserved. Layer networks have been trained with significant levels of noise to reflect the variability of average spiking rate of real neurons. The resulting trained network spontaneously learns to utilize all of its resources by creating clusters of similarly connected units that represent what were single units in smaller networks. These clusters presumably help to overcome the effects of noise by averaging. Networks made up of spiking units, with biologically based parameters and connections determined by the continuous network, are being studied. Their interspike interval distributions model those found in the data. The attractor dynamics of spiking networks, and the implications of those dynamics for perturbed neurons in such a network, are explored as a guide to further experimental testing of the recurrent model of dynamic short-term memory.
438.3 EXPRESSION OF 5'-ECENTONUCLEOTIDASE AND PHOSPHATASE ACTIVITY FROM THE OLFACTORY ORGAN OF THE SPINY LOBSTER IN XENOPUS OOCYTES. H.G. Trajano-Rosenthanal, M.A. Holman, R.M. Greenberg, R.A. Giusti and W.E.S. Carr* Whitney Laboratory, University of Florida, St. Augustine, Florida 32086.

The olfactory organ of the spiny lobster, Panulirus argus, consists of a dense array of aesthetasc sensilla on the lateral filament of the antennae. Electrophysiological studies have shown that sensilla include populations of receptor cells that respond to the odorant adenosine 5'-monophosphate (AMP). Biochemical studies have shown that sensilla also contain a potent extracellular enzymatic activity that rapidly dephosphorylates this odorant. In this study, we report that messager RNA (mRNA) isolated from lobster olfactory organ can induce the expression of 5'-ecto-AMPase/phosphatase activity in oocytes of the frog Xenopus laevis. Oocytes microinjected with this mRNA from lobster’s lateral antennular filaments exhibited an increase in their ability to dephosphorylate exogenous AMP, relative to non-injected and water-injected control oocytes. The amount of ectoenzymatic activity expressed depended upon the amount of lobster mRNA injected. Injection with non-messenger mRNA did not enhance the ability of oocytes to dephosphorylate AMP. Heterologous expression in Xenopus oocytes may be of general value for studying odorant-degrading enzymes, since the biochemical assays used to monitor expression are both simple and highly sensitive. This work was supported by grants from the National Science Foundation (BNS-8908340 and BNS-8805085) and the University of Florida (D-50-8999 and RDA-1-9).

438.5 MODEL FOR THE DISCRIMINATION OF ODORS BY 7-TM DOMAIN RECEPTORS AND BY A GROUP OF OLFACTORY NEURONS. P. Petet,1 I. Hermans-Borgmeyer,2 G. P. Gasic,3 M. E. Pellerin1, V. Biolcati1, G. B. Gasic,2 G. P. Gasic3, and J. E. Pellerin1.

Using PCR amplification, we recently cloned a novel multigene family of putative olfactory receptors characterized by 7 transmembrane (7-TM) domains and expressed only in olfactory epithelium. Experiments are in progress to determine whether the proteins encoded by these clones represent functional olfactory receptors which play a key role in the detection of odorants. The distribution of these putative receptors probably constitutes an integral part of the molecular mechanisms involved in discriminating among hundreds or even thousands of different smells.

In situ hybridization analysis with some of these putative olfactory receptors revealed patterns of expression that correspond to groups of olfactory neurons topographically distributed within the olfactory epithelium. We have named these patterns “primary olfactory maps.” We predict that the total number of primary olfactory maps is not infinite mainly because of the overlapping distribution of individual olfactory 7-TM receptors in the epithelium.

Currently, we propose a model for the discrimination of an odorant in which a given odor activates a group of olfactory receptors as well as a group of olfactory neurons. The combination of these two interactions produces “odorant fingerprints” consisting of a pattern of specific depolarizations that are sent to the CNS and further processed by the olfactory bulb.


The olfactory mucosa may receive extrinsic innervation from 3 sources: terminal nerve, trigeminal nerve, and autonomic nerves. Each of these nerves projects to the olfactory sensory neurons. Based on cell structure, staining pattern, and localization of immunoreactive elements, we have correlated VIP-immunoreactive innervation with its neural source. Immunofluorescence was used to identify and localize VIP immunoreactivity. Terminal nerve innervation was characterized by granular staining in occasional bipolar cells with thick, non-varicose processes localized within somatic mucous cells and their processes in the base of the epithelium, and in the olfactory sensory neurons. Trigeminal nerve innervation was associated with agranular staining in thin, varicose fibers in the region of the basal lamina, located at the base of the lamina propria, whose branches terminated within glands and blood vessels. Immunoreactivity for substance P was also present in fibers of these nerves, but was not colocalized with VIP. Thus, VIP-immunoreactive innervation from different neural sources is associated with different structures and functions in the olfactory mucosa. Supported by NSF BNS-88-21074 (MLG) and NIH DC-00159 (TGG).

438.8 VOLTAGE-SENSITIVE DYES LOCALIZE TRANSDUCTION EVENTS WITHIN OLFACTORY RECEPTOR NEURONS. K.C. Costelma, J. Brouwer2 and P. Farmer1, Anatomy & Cell Biology, Univ. of Cincinnati Medical Center, OH 45267.

Voltage-sensitive dyes confirm whole-cell patch recording evidence that transduction processes differ in frog and in salamander olfactory receptor neurons. Patch-clamp studies show that in frogs the dendritic and somal membranes are inactivated at rest, with unusually low conductances. In salamanders sodium channels are available for excitation and respond to stimulation with increases in sodium conductance. Voltage dye measurements in slices of olfactory epithelium in the laser scanning confocal microscope discriminate voltage gradients they show that the influx of calcium is an effective stimulus depolarizes only receptor cilia and the knob on the apical end of the dendrite in the frog. The membrane potential of the dendrite is unchanged, within the sensitivity limits of the technique. In salamander olfactory receptor neurons, stimulus-induced depolarization invalidates the dendrite and soma. It appears that the receptor cilia are the sites at which depolarization is generated. In the frog, the receptor cilia to the axon for action potential initiation through the cytoplasm without significant electrotonic spread. In the salamander, both electrotonic and active processes conduct the stimulus message from the cilia to the axon spike generator. In epithelium preparations viewed in face odor responses of different neurons are more closely compared to allow studies of the stimulus selectivity among neuronal populations. These results are consistent with recently developed methods that allow the use of voltage-sensitive dye microscopy. Supported by NIH grants DCB0342 and DC03477, University of Cincinnati Research Challenge and the Mark P. Herschede fund.
POSTSYNAPTIC RESPONSES IN BASAL CELLS ARE ELICITED BY STIMULATING RECEPTOR CELLS IN NECTRUS TASTE BUDS. Douglas A. Ewald & Steven Roper Dept of Anat & Neurobiol, Colo St U. Ft Collins CO 80523 & the Rocky Mtn Taste & Smell Ctr, Denver CO 80262

Receptor cells comprise the majority of cells (90%) in Necturus taste buds. Receptor cells extend apical processes into the taste pore. Basal cells which comprise only 10% of the total population, lack apical processes yet are involved in the majority of the responses in the taste bud (Delay & Roper, J. Comp. Neurol. 277: 268, 1988). In thin (300-500 μm) slices of lingual epithelium in vitro, responses to specific receptor cells with locally applied KCl (140 mM) elicited receptor potentials in receptor cells and long-latency depolarizing responses in cells that were subsequently identified by dye injection as basal cells (Ewald & Roper, Neurosci. Abstr. 16: 25, 1990). We have made simultaneous recordings from receptor cells and basal cells in the same cell pair to study the long-latency responses and test whether they represent synaptic coupling from receptor to basal cell. Varying the magnitude of the KCl-evoked receptor responses revealed a threshold for basal cell responses of a membrane potential between -20 to -10 mV. In about 20% of the simultaneous impalements, decremental stimulation of receptor cell elicited small depolarizing responses in the basal cells. The magnitude of basal cell responses evoked by electrical stimulation of a single receptor cell mean ± S.E.M. = 0.52 ± 0.06 mV, N=10) was 14% of the responses elicited by KCl stimulation of the entire population of receptor cells. The threshold for electrically-evoked responses was in the same range as that for elicited by chemical excitation.

The lack of a long latency in responses elicited by electrical stimulation suggests that the long latency in response to KCl stimulation resulted from the time for the chemically-evoked receptor potential to reach threshold. The incidence (20%) of synaptic coupling between receptor cells and basal cells and the ratio of amplitudes of direct, electrically-evoked responses to KCl-evoked responses (14%) suggest that basal cells might be plastic. The role of this type of synaptic connection in integrating or modulating the response to gustatory stimuli is currently under investigation. Supported by NIH grants DC02344, DC03734 and AG06557.

A. Frautschy and A. Baird, Dept. of Neurosciences, UCSD, La Jolla, Ca. 92037 and Dept of Cellular and Molecular Biology, Whiter Institute, 9894 Geneve, La Jolla, Ca. 92037.

Because they have not been directly induced in an animal model, the significance of beta-amyloid deposits in Alzheimer's disease remains unclear. Although recent data suggest that injection of solubilized beta protein and related peptides may have acute toxic or pharmacological effects, there is no data on existence of or levels of soluble beta protein in normal or Alzheimer brain making it difficult to assess their possible physiological or pathological significance. We have cloned G proteins from rat cortex and hippocampus and similarly isolated control lipofuscin fractions on the contralateral side. Rat brain sections were silver and immunohistochemically stained to study the proteolytic processing events and aggregational properties of APP and its fragments in excitatory and inhibitory neurons.


In Alzheimer's disease the typical amyloid deposits are mainly composed of beta-amyloid although being synthesized as part of a larger precursor protein (APP) from which it is released by so far unknown proteolytic breakdown events. We have been able to clone the histidine rich proteolytic mechanisms by which beta-amyloid APP fragments are generated and deposited in the brain. Expression plasmids coding for various C-terminal fragments of APP which include the B/A4 region were introduced into mammalian cell lines. The corresponding gene products were analyzed with antibodies directed against the B/A4 region and the cytosolic domain of APP. The C-terminal fragments of APP are proteolytically processed giving rise to a complex pattern of breakdown products. Fragments that react with β/amyloid antibodies accumulate within the cells and form aggregates that are not soluble in SDS buffers. Immunostaining of transfected cells reveals the presence of dense aggregates in the cytoplasm. These structures are β/amyloid positive and can be stained with Thioflavin S, suggesting that the aggregates adopt the characteristic β-pleated sheet conformation of β/amyloid. The accumulation of stable β/amyloid APP fragments within cells offers the possibility to study the proteolytic processing events and aggregational properties of APP and its fragments in excocytic cells.

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A SERINE PROTEASE FROM MONKEY AND ALZHEIMER'S BRAIN AND A CYSTEINE PROTEASE FROM ALZHEIMER'S BRAIN DEGRADE THE AMYLOID PRECURSOR PROTEIN (APP). C. R. Abraham, B. Razzaboni and G. ASEINE PROTEASE FROM MONKEY AND ALZHEIMER'S BRAIN AND A

...activities from AD brain. AD brain homogenates were fractionated and inhibitor (exposed to X-ray film. Two distinct active fractions were further labeled products were separated by thin layer chromatography and purified and characterized by using a battery of protease inhibitors. One activity appears to be a calcium-activated serine protease which cleaves the peptide between the methionine and aspartic acid. A second proteolytic activity was purified from saline-perfused, fresh-frozen monkey brain. The calcium-activated protease is also able to degrade the full length B-APP (a gift from S. Sisodia, E. Koc and R. Siman). The first protease(s) which generate the B-APP can lead to the design of protease inhibitors aimed to arrest amyloid deposition which is believed to cause neuron cell death and dementia. Supported by NIA, Alzheimer's Association and Cephalon, Inc.

CHARACTERIZATION OF NEURITE-STIMULATING ACTIVITY FROM CONDITIONED MEDIA OF TRANSFECTED PC12 CELLS THAT OVEREXpress 8/4-AMYLOID. R.E. Majocha, B. Tate, M. Ventosa-Michelman and C.A. Marotta, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

...activity. Abs to B-amyloid are only partially effective in removing activity. These results suggest that multiple stimulatory factors are released from PC12 cells overexpressing 8/4-amyloid. Supported by NHI AG01126 and the Metropolitan Life Foundation.

B-AMYLOID PRECURSOR PROTEIN EXPRESSION IN RAT BRAIN. INCREASED LEVELS OF KUNITZ DOMAIN CONTAINING FORMS AFTER NEURONAL LESION. G. Mengden 1,2, C. Solà1, J.M. Palacios 1, Y. Li 3, C. Probst 3 and J.M. Palacios 1.

We have examined the effects of neuronal lesions in amyloid expression in the rat brain. Two types of neuronal lesions were induced in rats 1) ivc injection of kainic acid and 2) anatomy of motor neurons of cranial nerves. The effect of these lesions on the expression of the different forms of the B-amyloid precursor protein (BAPP) were examined using in situ hybridization histochemistry (ISHH) with oligonucleotide probes specific for each form and by immunohistochemistry with antibodies raised against different synthetic peptides of the BAPP.

Kainic acid injections resulted in neuronal cell death and glial reaction in the hippocampus. As previously described (Simas et al., 1989) sizable cell death is displayed post-kainic acid (PcA) BAPP. The ISHH experiments revealed a loss of BAPP concurrent with the Kunitz domain containing forms 751 and 770 were dramatically increased in the region. Axotomy of the facial and hypoglossal motor nerves resulted in increased BAPP immunolocalization in these motor neurons shortly after axotomy.

The mRNA coding for the different BAPP forms were also increased. After regeneration, the level of BAPP mRNA and protein returned to normal. These results suggest different modes of response of the BAPP protein to neuronal injury that affect differentially the forms containing the protease-inhibitor domain.

8/4/AMYLOID PRECURSOR PROTEIN IN ALZHEIMER'S PAIRED HELICAL FILAMENTS. F.P. Zemlan, G.E. Dean and G.D. Vogt, Alzheimer's Research Center, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

Several laboratories have demonstrated that antibodies (Abs) against restricted regions of the 8/4 amyloid precursor protein (APP) label neurofibrillary tangles in Alzheimer's disease (AD) brain. The present study examines whether APP is loosely associated with paired helical filaments (PHF) in tangles or is directly incorporated into PHF core protein. Alzheimer's PHF was purified as previously reported by our laboratory, then examined on immunoblots with Abs raised against recombinantly expressed APP. Anti-APP500-695 selectively labeled the monomeric 66 kD PHF core protein (AD) on immunoblots while anti-APP20-304, anti-APP44-592 and anti-KPI did not. As APP500-695 includes the C-terminal end like the native APP protein. Recombinant APP751 and APP770 formed complexes with epidermal growth factor binding protein (EGF B) whereas APP695 did not. In addition, recombinant APP751 and APP770 label intracellular and extracellular epitopes whereas APP695 did not.

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THURSDAY AM

ALZHEIMER'S DISEASE: AMYLOID II

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439.11 MUTATIONAL ANALYSES OF AMYLOID PRECURSOR PROTEIN-LIKE GENE IN DROSOPHILA. L. Luo and R. White, Department of Biology, Brandeis University, Waltham, MA 02254.

The Drosophila Amyloid precursor protein-like gene encodes a protein with structural homology with the mammalian biochemical properties and tissue distribution compared with APP955, a neural-specific form of the amyloid precursor protein associated with Alzheimer disease (Rosen et al., Proc. Natl. Acad. Sci. USA 87:12478-12482, Luo et al., Nature 384:3890, 1990). We are using a genetic approach in Drosophila to investigate the in vivo function of this class of molecules. By deletion mutagenesis, a null allele of the gene was generated and App(+) flies by the criteria of PCR, Southern and Western analyses. These App(+) flies are viable and morphologically normal, however, they exhibit behavioral deficits. A phototaxis behavior assayed by a counter current immobilization choice was chosen for further analysis because the quantitative difference could be partially resolved by transduced heat-shock promoter-App(+) cDNA construct. This behavioral abnormality provides a potential assay for App(+) function. We have also generated by in vivo mutagenesis constructs expressing secretion-defective or constitutively secreted APPL proteins. These constructs were injected into flies through germ-line transformation. Such transduced genes, when introduced into App(+) background, may provide insight about which form is biologically active by their ability to rescue the behavioral defects. In addition, these cells may be used to test the biochemical properties of various forms of APPL protein in developing nervous system.


Previous studies have shown the accumulation of amyloid and the presence of a variety of different unlabelled ligands in Alzheimer's disease (AD) brain. In the present study the potential binding interaction between different PGs and the extracellular domain of the BAP was explored. In situ and affinity column chromatography experiments utilizing isolated [35S]-sulfate labelled PGs from medium or cell layers of bovine aortic endothelial cells or smooth muscle cells, demonstrated that a high Mr heparin (Mr > 600 kD) binds relatively tightly, 2 smaller heparin sulfate/chondroitin sulfate (DS/CS) PGs (Mr ~ 100-250 kD) bind weakly, and a large chondroitin sulfate PG (of smooth muscle cells) did not bind to the BAP. Competitive inhibition studies using [35S]-labelled heparin in the presence of a variety of different unlabeled ligands suggest that both the core protein and GM1 chains of the BAP are involved in BAP binding. The specific binding of the high Mr heparin to the BAP may explain its constant co-localization to BAP amyloid deposits in AD brain, and further implies a possible role for this particular PG in BAP accumulation in AD.

440.1 RESPONSES TO CLICKS OF THE CHINCHILLA BASILAR MEMBRANE. M.A. Ruggiero, N.C. Rich and A. Recio, Dept. of Otologyngology, Univ. of Minnesota, Minneapolis, MN 55444.

Basilar membrane responses to clicks and tones were studied in chinchilla cochleae using a new application of laser velocimetry (Ruggiero and Rich, Hearing Research, 51:210-230, 1991). Basilar membrane velocity at a site located 3.5 mm from the round window [characteristic frequency (CF): 8-10 kHz] was determined from the Doppler frequency shift of laser light reflected from glass microbeads (10-30/~m) placed on the basilar membrane. Responses to clicks in healthy cochleae consisted of relatively undamped transient oscillations with periodicity close to 1/CF. Intense rarefaction clicks caused initial basilar membrane motion toward scala vestibuli after a latency of about 90 microseconds (measured from the onset of inward stapes displacement). The initial response oscillations grew linearly with stimulus intensity but later response cycles grew nonlinearly, at rates less than 1 dB/oct. Thus, with increases in stimulus level, the response envelopes became progressively more asymmetrical, with maxima shifting to earlier times. The frequency spectra of click responses were computed by Fourier transformation. As click level increased, sharpness of frequency tuning diminished and the maximal spectral response component shifted to lower frequencies. Spectral components immediately below CF phase lagged responses evoked by pure tone stimuli. These responses to clicks were observed to tone-evoked responses by less intense clicks, while those just above CF led lower-level responses. In spite of the CF-specific nonlinearity, the magnitude and phase frequency spectra of click responses resembled those for responses to tones. Upon cochlear damage or death of the chinchilla, all nonlinearities disappeared; the gain spectra became broadly tuned and insensitive and thus very similar to those of responses evoked by intense stimuli.


The mammalian auditory nerve is composed of thick, myelinated axons from type-I neurons and fine, unmyelinated axons from type-II neurons. Type-II axons number only 5% of all axons in the inner ear, and their sound-evoked responses have not been documented. Since their fine axons should have long impulse conduction times, type-II units recorded peripherally in the spiral ganglion should have long antidromic latencies to shocks from a stimulating electrode placed centrally in the auditory nerve root. Single units were recorded with micropipettes from the basilar-turbin spiral ganglion of the anesthetised guinea pig. Most units (n = 129) had short-latencies (0.3-1.0 msec) and shocks and sound-evoked activity typical of type-I units (Liberman, 1982, Science, 216:1239-1241). A second class (n = 24 units) had sound-evoked activity typical of olivo-cochlear efferent fibers (Brown, 1989, Hearing Res. 40:93-110); and sometimes responded to high-level shocks with latencies of 1.5-2 msec. Presumably at high shock levels, the current spreads to the nearby vestibular nerve where olivo-cochlear fibers run. A rare class of units (n = 5) had much longer latencies (2-3 msec), appropriate for the calibre of type-II axons (Brown, 1987, J. Comp. Neurol. 260:591-604). The former two classes had moderate to high electrical thresholds. One long-latency unit (from a preparation with good type-I thresholds) responded to high-level noise bursts. It may be suggested that these long-latency units correspond to type-II neurons, although an unusual type of olivo-cochlear neuron cannot be entirely ruled out.

440.3 GLUTAMATE RECEPTOR mRNA EXPRESSION IN THE RAT COCHLEA. A.F. Ryan, D. Bruemmer, M. Kraft, Dept. of Otolaryngology and Department of Neurosciences, UCSD Medical School and VA Medical Center, La Jolla, CA 92030.

Pharmacological evidence suggests that glutamate may be the transmitter between cochlear hair cells and afferent neurons. Recently, a gene family encoding subunits of non-NMDA glutamate receptors has been cloned and sequenced (Hollmann et al., Nature 342:643, 1989; Boulter et al., Science 249:1032, 1990). To determine whether these receptors participate in cochlear neurotransmission, adult rat cochleas were screened for expression of the genes GluR1, GluR2, GluR3 and GluR4. Hybridization. GluR1 riboprobes showed no hybridization with any cochlear cells. GluR2 and GluR3 riboprobes both showed intense hybridization with spiral ganglion neurons (SGNs), consistent with a major role for these receptors in SGN neurotransmission. GluR4 riboprobes showed positive but lower levels of hybridization to SGNs. The most likely site for GluR2, GluR3 and GluR4 receptor subunits is on SGN afferent dendrites beneath the cochlear hair cells, since presynaptic endings are relatively sparse on inner hair cells in cochlear nucleus and there is no evidence that the olivo-cochlear efferents use glutamate as a neurotransmitter. These results support the hypothesis that glutamate acts as a neurotransmitter between cochlear hair cells and SGNs. Supported by DC00139 and the VA Research Service. Glutamate receptor cDNAs were provided by J. Boulter of the Salk Institute.

440.4 ANATOMIC DISTRIBUTION OF CHOLERA TOXIN BINDING SITES IN THE COCHLEA. P.A. Santi and P. Mancini, Dept. of Otologyngology, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

The B subunit of cholera toxin specifically binds to the carbohydrate residues of the GM1 ganglioside. A FITC-labelled B subunit of cholera toxin was applied to fixed, cryosections of the mammalian cochlea in an attempt to localize the GM1 ganglioside within cochlear tissues. The cholera toxin probe strongly reacted with cells of the organ of Corti, interdental cells, basilar membranes, internal and external sulcus cells, spiral prominence cells, intersolubul globuli, and cochlear nucleus cells that extend into the internal auditory canal. Less reactivity was observed in cells of the stria vascularis, spiral ganglion, modiolus, and Reissner's membrane. No reactivity was observed in the connective tissue cells of the spiral ligament, spiral limbus, basilar membrane or perilymphatic scalae. Controls consisted of probe binding to normal brain tissue, absorbing out the probe with bovine GM1, binding inhibition of the probe with unconlabeled cholera toxin, and ethanol extraction of GM1 from cell membranes prior to probe exposure. The functional significance of cholera toxin binding sites and their relationship to the GM1 ganglioside in the cochlea will be discussed. (Research supported, in part, by NIDCD and DRF.)

To determine the potential role of the ODC/polyamine system in the functional development of the rat cochlea we quantified ODC and polyamines in developing cochlear tissues and then determined the effects of postnatal DFMO treatment on both cochlear function and on cochlear polyamine metabolism. DFMO, an inhibitor of ODC, is a useful biochemical tool in studying polyamine-dependent processes. Rats (n=3 to 5) were terminated at the postnatal ages ranging from 3-60 days and cochlear tissues were analyzed for ODC activities. Cochlear ODC increased rapidly during the first 10 days of life, peaked on days 9-10, then rapidly declined. ODC in the lateral wall-organ of Corti was greater than in the cochlear nerve in developing, but not in adult rats. Polyamines were elevated during this period of differentiation of various cell types comprising the lateral wall-organ of Corti. The spermidine/spermine ratio was high in the cochlear nerve, even in adults, indicating the continued elevated level of protein and nucleic acid synthesis necessary for nerve function. This may explain the reversibility of DFMO ototoxicity in humans, since the ring is cytotoxic and not neurotoxic. Rats treated with DFMO (500 mg/kg/day) during the period of increased polyamine biosynthetic activity demonstrated age- and frequency-dependent deficits in outer hair cell function, as assessed by acoustic distortion-products. DFMO significantly inhibited ODC during this specific period. The developing rat appears to be hypersensitive to the effects of DFMO during the period when polyamine biosynthetic activity is increased. We are currently evaluating the effects of DFMO on cochlear polyamine levels to correlate biochemical effects with observed electrophysiological data. The polyamines are critical for the normal development of cochlear function.

SENSITIVITY OF AUDITORY NERVE FIBERS TO THE RELATIVE PHASE OF FREQUENCY COMPONENTS IN COMPLEX SOUNDS. D.A. Bodnar and D.G. Drescher. 1Dept. of Otolaryngology, Wayne State University School of Medicine, Detroit, MI 48201. 2Laboratory of Biochemistry, Wayne State University School of Medicine, Detroit, MI 48201.

The presence of mineralocorticoid (Type I) receptors in the mammalian inner ear was previously suggested by aldosterone modulation in vivo of H-ovalbumin binding measured in vitro (Ploksni et al., Assoc. Res. Otolaryngol. Abstr. 13: 303, 1990). We have now measured in vivo in the lateral wall of the basal turn of the cochlea and in the ampulla of the semicircular canal, respectively. [3H]-Aldosterone binding has now been measured in vivo in the lateral wall of the basal turn of the cochlea and in the ampulla of the semicircular canal in male Hartley guinea pigs. Microdissected inner-ear tissue was assayed with [3H]-11-deoxycorticosterone, and binding determined. Specific binding was defined as total binding in the absence (experimental) minus binding in the presence (control opposite ear) of 300-fold excess of unlabeled hormone. Binding sites were also corrected for [3H]-11-deoxycorticosterone present intercellularly, monitored with 14C-cortisol and normalized to tissue dry weight. Specific binding, thus measured, was 9.1 ± 1.5 (g) and 9.8 ± 1.5 (f) pmol per mg dry tissue [mean ± SEM (n)] in the lateral wall of the basal turn of the cochlea and in the ampulla of the semicircular canal, respectively. A complex sound is uniquely specified by the magnitude and phase spectrum of individual frequency components. Examinatory tuning curves of individual auditory nerve fibers only reflect the selectivity to single tone stimuli. Thus, tuning curves do not provide any information regarding the encoding of the relative phase spectrum in more complex signals.

To address the question of peripheral sensitivity to phase spectrum, we recorded from single units in the VIIIth nerve of the bullfrog (R. catesbeiana) using multi-tone stimuli. These stimuli consisted of the first 10 components of a harmonic series with a fundamental frequency ranging from 0.1 to 400 Hz. Each waveform spanned most of the frequency range of the bullfrog's vocal repertoire. For all units the same standard reference stimulus was initially presented and then the relative phase of single harmonic components was varied. Most of the cells exhibited pronounced changes in their spike rates when the relative phase of only a single component was varied, and were not sensitive to changes in phase of the component closest to their best excitation frequency. Peaks of spike rate vs. phase angle revealed a wide variety of patterns in spike rate changes. Most cells also showed significant changes in their coefficients of synchronization to the fundamental period of the stimulus. The shapes of cycle histograms, plotted on the fundamental period, changed with shifts in the relative phase of a single harmonic.

The phase relationships of individual frequency components affect the fine temporal structure of the acoustic signal waveform. The observed sensitivity of auditory nerve fibers to these relative phases suggests that complex waveform features of auditory stimuli are encoded by the peripheral auditory system.

This research is supported by NIH grant NS 09244.
441.1

**Magnitude measurement of hindlimb withdrawals to graded noxious heat in conscious rats.** R.L. Mitchell, D. Ansley* and E. Carstens. Dep. of Animal Physiology and Veterinary Anatomy, Univ. of California, Davis, CA 95616.

Most animal pain models measure the latency (i.e., the time to onset of a reflex), but qualitative responses may also provide useful information. However, whether measurable response of magnitude would be desirable in showing psychophysical stimulus-response relationships throughout the nociceptive system, we measured the magnitude of the conscious rat's hindlimb flexion withdrawal reflex.

Sprague-Dawley rats were habituated to a restrainer through which the left hindlimb protruded. The ventral hindpaw was taped against a Pettier thermometer. Hindlimb flexor EMGs during attempted withdrawals elicited by graded noxious heat pulses (40-50°C, 5 s) were monitored by telemetered microwires in biopsic femora. Responses were quantified (area beneath integrated EMG for 7 s after heat onset) and normalized to 100. Humans tolerated all stimuli and rats showed no signs of distress.

Integrated EMGs increased in a graded fashion from 42-52°C (9±12); no rats withdrew at 50°C. The slope of the stimulus-response function was reduced, with no threshold change, 20-40 mm after systemic administration of morphine (3.5 mg/kg i.p.) in a naloxone-resistant manner. This relatively simple method measures reflex magnitude from threshold to tolerance levels of noxious heat, is sensitive to the tail withdrawal, and might therefore serve as a useful new quantitative pain model.

**441.2**

**Quantification of the rat tail-flick reflex (TFR).** E. Carstens and C.G. Wilson*. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

The TFR is a widely used pain test measuring the latency for a rat's tail to be flicked away from an unregulated noxious heat source. However, it is unknown if TFR magnitude increases in a graded or ballistic way with stimulus intensity, cluing the interpretation of all-or-none increases in TFR latency (threshold)? often seen following analgesia. We therefore developed a method to measure TFR force in conscious rats, whose radially arranged transducers measured the force of horizontal, dorsal and rostrally directed components of TFRs elicited by feedback-controlled noxious radiant heat pulses (40-50°C, 5 s) delivered unilaterally to the tail. Areas beneath force traces were integrated to calculate TFR force vectors. Stimulation evoked no signs of distress.

The rostral force component of TFRs was much larger than horizontal/dorsal components. Most rats showed a preferential horizontal direction of TFRs (left or right in approximately equal numbers regardless of the side of tail stimulation). TFR force vectors increased linearly from 40-45°C and then leveled off (N=10). Responses to 44°C stimuli decreased over repeated trials (2 min interval) and recovered following a 15 min break. Responses to repeated 50°C stimuli did not decrease. Thus, the TFR is a reflex response to graded ranges of noxious heat intensities and may habituate at low temperatures.

**441.3**

**Ultrastructural and neurophysiological analysis of the internal architecture of intact human nerves.** R.G. Ultrasound and N. Carstens. Dept. of Neurophysiol, Hudding Univ Hospital, 141 86 Huddinge, Sweden.

Single units within the entire nerve fibre spectrum can be studied in man. Furthermore, thin concentric needle electrodes are available as a tool for an integrative analysis of both functional and structural aspects of human peripheral nerves.

In this study, we used concentric needle electrodes (O.D. 200-250 μm, I.D. 0.70 μm) for percutaneous median nerve exploration. The firing characteristics of A and C fibres and their relative localization within the nerve were studied in four different recording sites in the nerve. Electrodes with thinner cores were used to screen intraneuronal fascicular organization, individual fascicles destined to the hand were mutually organized in a somatotopic manner at both wrist and elbow level. Electrodes with a thin core were used to test the inner core of a fascicular fibre organization. Many observations suggested the presence of an orderly organization also within the individual fascicle, which extended to the single unit level.

The traditional view that the nerves are spread out in diameter proximally in limb nerves was not substantiated by these studies. Instead we feel tempted to suggest that our data reflect the existence of a peripatetic somatotopic organization in the periphery of the neuraxis and then extend to central levels of the nervous system. Some clinical implications of the obtained data will be discussed.

**441.4**

**The failure of conduction of impulses in long range afferents running in rat spinal cord.** PD Wall and SB McMahon. Dept Anatomy, University College London, UK, and Dept Physiology, ST Thomas' Hospital Medical School, London, UK.

When myelinated sensory afferents enter the spinal cord, they form a T branch running rostrally and caudally. The rostral branch may run as far as the dorsal column nuclei. However, it may also run for many segments and extend with dorsal horn terminals far beyond the region in which such afferents have been shown to produce postsynaptic effects. Lower thoracic segments (T5-T12) and their corresponding dorsal horn terminals do not normally conduct orthodromic impulses by measuring their partial refractory period either following local microelectrode stimulation in the dorsal column or after a single injection of an orthodromic impulse from the dorsal root. This measure showed that orthodromic impulses may fail to penetrate the dorsal horn at distant caudal sites in all fibres tested. In contrast, the rostral branch always appeared to conduct orthodromic impulses. Furthermore, this apparent orthodromic blockade is relieved 2-10 days after the neighbouring dorsal roots have been sectioned.

**441.5**

**Activity of neurones in the rostroventral medulla which respond to noxious heat are correlated to changes in arterial blood pressure.** G. Thurston & A. Randich. Dep. of Psychology, Univ of Alabama at Birmingham, Birmingham, AL 35294.

Extracellular recordings were made of neurones in the rostroventral medulla (RVM) in methohexitol-anesthetized rats. The neurones were classified as ON, OFF, or OFF-ON and NK neurons depending on their response to noxious heat applied to the tail. ON neurones were excited just prior to the tail flick (TF) and OFF neurones were inhibited just prior to the TF. Both ON and OFF cells showed spontaneous fluctuations in activity that were correlated with fluctuations in arterial blood pressure (ABP). When ON neurones are active and OFF neurones are inactive, ABP is lower than when OFF neurones are active and ON neurones are inactive. Further, changes in neuronal activity precede changes in ABP. In addition, electrical stimulation of the cervical vagus produces a depressor response at intensities which increase ON cell activity and decrease OFF cell activity. These data suggest that ON and OFF cells in the RVM are important in autonomic regulation.

Supported by NIH grants NS24958 and NS22966.

**441.6**

**Coactivation of the nucleus raphe magnus (NRM) by lateral hypothalamus (LH) and the periaqueductal gray (PAG).** M.M. Bebeacu, M. Jiang and S.D. Chesslin. Dept of Physiology and Biophysics and Anesthesia, U. of Cincinnati, Cincinnati, OH 45267-0576.

There is evidence that NRM receives input from both the LH and PAG. However, it has not been established whether a given cell in NRM responds to stimulation of both sites. We tested this possibility by extracellular and intracellular recording from NRM neurones and measurement of their response to electrical stimulation of LH and PAG. In addition, we examined the role of neurones (NT) in coactivation of NRM. Extracellular recording was made from 100 NRM neurones at adult male rats; in 10 cells intracellular recordings were made. Cells were identified according to their response to peripheral stimulation. Stimulation of both sites excited 15 cells and兴奋 12 cells 29 cells responded in opposite direction to LH/PAG stimulation. The excitatory response to PAG and LH had an onset latency of 24.5±4.4 and 14.9±9.8 ms respectively. The inhibitory response had an onset latency of 14.7±8.34 and 11.4±10.12 ms and duration of 9.5±3.16 and 62±25.45 ms respectively. In 2 out of 3 cells recorded intracellularly, IPSPs produced by stimulation of LH had a larger magnitude and duration than IPSPs produced by stimulation of PAG. In 3 cells the effect of NT on NRM was tested; 31 cells were excited, 14 were inhibited and 11 were unresponsive to NT. Comparison of the response of neurones to LH stimulation showed no significant interaction. It is suggested that 60% of neurones in NRM are coactivated by PAG and LH, 2 the inhibitory response is more dominant and the other 3 NT is unlikely to be the mediator of the coactivation response. Supported by PHS grant NS20643.
441.7

Raccoon thalamic neurons with receptive fields (RFs) totally (N = 10) or partially (N = 10) on glabrous skin of the forepaw were examined for their responsiveness to various mechanical and thermal stimuli. Recording loci were in the core of the ventrobasal complex (VB; N = 8), the ventral posterior lateral (VPL; N = 3), and the medial division of the posterior nuclear group (POM; N = 9).

Eleven VB neurons were classified as multimodal (RFs) and one as nociceptive specific (NS). All VB neurons were as NS and three as NS. All NS neurons had high threshold RFs than low threshold RFs, and this difference often was greater than for VB neurons. 85% of the total were rapidly adapting and 14% slowly adapting.

For 16 out of 19 neurons tested, the thermal threshold was greater than 53°C. Thermal thresholds were between 51°C and 53°C in three NS units. Sensitization following a 30 s burn (53°C for 30-60 sec) to the RF, persisting for up to one hour, occurred in four of eight units tested.

These data indicate that raccoon thalamic cells responsive to nociceptive input are not segregated within VB, but are located throughout this nucleus as well as in POM. Many of these cells may be sensitive following an injury, and thus contribute to hyperalgesia. [Support: NS-1946, USPHS.]

441.9

We have previously reported morphological feature of primary sensory afferent fibers which showed electrophysiological fast adapting, slowly adapting, and slowly adapting SA1-type afferents. In the present paper, we would like to submit more detailed quantitative analysis on their relation to morphological features of FA and SA types of primary afferents. Under sodium pentobarbital anesthesia, trigeminal primary afferent fibers of FA and SA were characterized electrophysiologically and they were stained with horseradish peroxidase and then observed with a light microscope. The terminal and en passant boutons of these fibers were observed under a microscope, and then analyzed three-dimensionally using computer. The densities of boutons in each trigeminal subnucleus were calculated by defining a volume of terminal arbor. The density of the terminal arbor showed no significant difference between that in the trigeminal principal nucleus (Vp) and that in the oral subnucleus of the spinal trigeminal nucleus (Vop). On the other hand, the density of SA type boutons in Vp were higher than that in Vop. The results suggested that FA- and SA-type primary afferents had characteristic features in terms of the density of boutons.

441.11

Earlier studies were made of cortical neural activity in waking M.multata discriminating between the frequencies of mechanical stimuli on glabrous skin on one hand. They projected the other to one of two targets to indicate whether the comparison stimulus of a pair was higher/lower than that of the base stimulus; e.g., 30 vs 32 Hz. Such stimuli evoke periodic activity in postcentral neurons (areas 3b and 1) related to Meissner afferents innervating the hand. The harmonics of response to trains reflect precisely stimulus frequencies; the neural discriminandum is the difference in the cycle lengths in the neural periodical order code.

Identical experiments have now been made in the motor and premotor cortical areas of the hemisphere opposite the projecting hindlimb area. Thal/1 cortex. A selective signal of the correct discrimination occurs during one or the other sets of comparison stimuli, often beginning about 200 msec after onset of the comparison stimuli and frequently continuing into the reaction time. The discharge is periodic. No similar responses were observed when similar stimuli were delivered to the hand of an alert but sedating monkey. We interpret this to be a post-discrimination signal projected trans-callosally from the contralateral sensory hemisphere to the motor & premotor areas.

441.8

Novel 19-channel MEG equipment was used to record somatosensory evoked fields (SEP) in normal subjects. The channels were arranged in a hexagonal configuration and were spaced between nearest neighbors being 2.5 cm. The SCQUIDs were connected to axial first-order gradiometers with 2.0 cm diameter and 5.0 cm baseline. All channels were in parallel. The exact relationship between the subject's head and the recording sensors was monitored using three 3-D coils placed on the scalp (international 10-20 locations Fz, Oz and Pz). The coils served as a defined magnetic source giving the coordinates of each coil relative to the gradimeters.

Somatosensory stimuli were electrical pulses of 0.2 ms duration given to the right median nerve at wrist. The stimulus intensity was adjusted above the individual motor threshold. 100 sweeps were averaged and a recording window of 200 ms was used. The bandpass was 0.5 - 250 Hz. The following components were identified: N20, M25, M80, M110 and M140. Phase reversal was observed for M20, M35, M80 and M110 components within one recording location posterior to C3. Adjuional locations were recorded for finding the maxima of the fields of interest. The current dipole estimations confirmed multiple generators in the somatosensory cortex. The estimated dipoles were superimposed on individual MR images of the subjects.

441.10

A model of a thalamocortical network (Neurosci. Abstr. 15:831, 1989) has been tested for its ability to simulate barreloid neurons' responses to different whisker stimuli. The model incorporates known anatomical and physiological features of a barrel in cortical layer IV and is actuated by actual pre-recorded spike trains from thalamic barreloid neurons.

Initially, responses of spiny and smooth neurons to deflection of the principal whisker with EMG alone were studied. Model parameters were adjusted to produce accurately the reductions, relative to thalamic input, in the sizes of spiny and smooth responses. The increases in these responses for smooth cells. Subsequently, the same model parameters correctly produced the disproportionately smaller ON responses evoked in spiny cells by adjacent whisker stimuli. Also in both cell types, movements of adjacent whiskers produced sustained post-excitatory inhibition and realistic response suppression to subsequent deflections of the PW. Finally, when the duration of the PW alone stimulus was increased, the simulated OFF response increased appropriately. Their circuits within a barrel are sufficient to produce significant changes in spatial and temporal characteristics of the thalamic input signals. Supported by NS19550 and the Pittsburgh Supercomputing Center.

441.12

Pertussis inhibition in sensory pathways is generally attributed to lateral inhibitory connections. While this is convincing in prethalamic pathways, the situation is less clear in the thalamus and neocortex. Thalamocortical circuitry is incompletely defined at present, and in some cases there is an apparent mismatch between observed inhibitory effects and intracortical inhibitory synaptic connections. This paper puts forth the hypothesis that a different mechanism, competitive distribution of activation, underlies some inhibitory effects observed in neocortex. Analysis of a mathematical model based on this hypothesis predicts that pertussis inhibition effects generally observed in lateral inhibitory connections can alternatively be explained by competitive distribution of activation in the absence of such inhibitory connections. Computer simulations verify these predictions by demonstrating Mexican Hat patterns of lateral interactions, transformation of diffuse activation into tightly localized patterns of activity, and edge enhancement. Analysis and simulations also show that the amount of inhibition can be adjusted by varying the intensity of the underlying competitive process. The concept of competitive distribution of activation provides an important alternative perspective for interpreting neocortical and thalamocortical circuitry and can serve as a guide for further morphological and physiological studies. For example, it provides an explanation for the existence of cortex-thalamus connections, and predicts that analogous circuitry must exist in neocortex.
442.1 MOTION OF CERVICAL VERTEBRAE DURING DIFFERENT HEAD MOVEMENT TASKS IN HUMAN. D.H. Wang, M. Graf, C. de Waele, P.P. Vidal and F. Gielen. The Rockefeller University, N.Y. 10021, Lab. Physiologie Neurosensorielle, CNRS, 75270 Paris, France; Schildau-Klinik, 3370 Seesen/Harz, FRG.

Our experiments, based on cineradiographic examination of human head movements in the sagittal plane indicated that head rotation results from simultaneous individual translations (sliding) of cervical vertebrae. In the present study we investigated the distribution of vertebral motion at different cervical levels during different head movement tasks. Subjects were asked to perform voluntary, supine, head movements. The cervico-thoracic and atlanto-axial vertebrae were measured from myelography images.

During voluntary, supine, head movements, translational movements of the cervical vertebrae were uniformly distributed along the segments. Thus, a global center of head rotation could be approximated around C6. During the nodding task, however, the upper segments participated to a larger extent. In this case, the global center of head rotation could be approximated around C3. Furthermore, significant degrees of pure rotation at the upper joints (C0 to C1, and Cl to C2) contributed to head rotation when the head reached extreme flexion and extension. This result suggests that the biomechanical constraints within the cervical spine (i.e., passive or effective of intervertebral muscles) may be the main factor for different kinematics of the two types of head movements studied. (Supported by NIH grant NY-0613)

442.2 FORCE CONTROL IN PRECISION GRIP: EXISTENCE OF SYNERGIES? M.A. Maier*, M.C. Hopp-Reymond and M. Meyer* Univ. of Zurich, Brain Research Institute, CH-8092 Zurich, and Dept. Neurology, CH-8091 Zurich.

Isometric force generation in the precision grip requires the delicate interaction of many muscles in timing and activation levels. We have quantitatively examined the participation of each muscle in force control and the putative existence of muscular synergies.

Subjects produced force between index finger and thumb with visual feedback in a ramp-and-hold paradigm with 3 consecutive force steps up to 3.0 N. Full-wave rectified and averaged EMGs of 15 muscles were used for computing correlations between muscle activity and force, as well as correlations and cross-correlations (CC) between the activity of muscles recorded simultaneously. The results are, that the majority of the thumb and index finger muscles increased their activity monotonically as a function of force, with considerable participation of some muscles. The putative existence of muscular synergies was confirmed, e.g., the index and thumb muscles showed muscular coupling in 50% of the pairs tested with some variability. Most prominent was coactivation (80%), and trade-off was rare (12%). Synergies between muscles of a single finger were almost twice as common as those between a thumb-and-index finger muscle pair. Temporal coactivation within muscle pairs was present in 20% of the cases, which all had clear CC peaks deviating no more than 8 ms from zero.

These data provide evidence, first for flexible muscle synergies that are not necessarily linked by biomechanical constraints, such as between flexor and extensor or between index and thumb muscles, and second for close synchronicity of a few muscle pairs, which in the majority are innervated by the same peripheral nerve.

442.3 SENSORY TRIGGERING OF A SEQUENCE OF POSTURAL RESPONSES P.J. Cordo, Cent. Medico Riabili, Veruno, Univ. Studi, Milano.

Sudden destabilization of the body center of gravity with respect to the support surface is known to trigger several postural responses. In the present study, we show that this short latency response is only the first of a sequence of responses and that this sequence is triggered by the nervous system to trigger each response in the sequence is different.

Seven normal humans stood on a posture platform and were translated forward or backward from 3 initial postures, leaning forward or backward, or standing erect, to enhance or reduce the destabilizing effect of the platform movement. Subjects were instructed to take a step only if absolutely necessary. When subjects reduced the effect of platform movement, they produced the well-known response in which the stretched ankle and knee muscles retracted the center of gravity back over the feet ("torquing"). When subjects stood erect, they first produced the torquing response and then rose up on the heels or toes to shift the fulcrum point ("rising"). When subjects enhanced the effect of platform movement, they performed the first 2 responses with a step ("stepping"). The first response is more likely triggered by somatosensory input—for thetorquing response, probably non-specifically related to the movement, and for the rising response, specifically related to change in ankle angle. The stepping response is related to the velocity of the center of gravity, and is more likely triggered by visual or vestibular input.


Standing subjects generated isometric tension by pulling upward on a bucket handle attached to one end of a cable with the elbow joint at 90° and the forearm horizontal and supinated. Other joints were braced to insure that the elbow flexors were the prime movers for the task. The moment arm of the isometric load was changed by anchoring the other end of the cable at different angles to the horizontal, thus altering the angle (Θ) between the cable and the forearm varied between 90° and 150°. The subjects made the same effort at each anchor point in a given trial, low-, medium- and high-effort were used in different trials. The torque/effort ratio for the elbow flexors [torque = cable tension (sin Θ)] was found to vary in a characteristic fashion, first increasing as the angle increased and then falling below the 90° value between 138° and 145°. Increases in torque/effort ratio were more pronounced when the effort was high and presumably reflect involuntary motor behavior designed to enhance cable tension until Θ is sufficiently large (>140°) that the line of action is toward the body. Supported by NIH grant NS 21972, NS 07172 and NS 07938.

442.5 DIRECTIONAL INFORMATION DURING SLOW GOAL-DIRECTED ARM MOVEMENTS. J.L. de Graaf (1), J.L. Penier van der Gon (2), A.C. Sittig (1) (SPON; European Neuroscience Association) (1) Product ergonomic, Delft University of Technology, Jaffalaan 9, NL-2628 BX Delft, The Netherlands, (2) Medical and Physiological Physics, University of Utrecht, Prinsepplein 5, NL-3584 CC Utrecht, The Netherlands.

Recently we have shown that when subjects are asked to make slow arm movements they start the movements to most targets in a direction that deviates consistently from the direction of the straight line between initial position and target position (de Graaf et al. 1991 Exp Brain Res 84: 434-438). To explore this phenomenon we did the following experiment with two conditions: the blindfolded and two sighted blindfolded subjects. The subjects were seated at a table. They were asked to make slow arm movements to proprioceptive targets (the index-finger of the other hand) at a distance of 30 cm. We analysed the initial movement direction as well as the curvature of the movement trajectories. The results show that 1. The sighted subjects as well as the blind subjects started their movements to most targets in directions comparable to the directions we found in earlier experiments. 2. And 2. The blind and sighted subjects showed more or less the same curvature in their movement trajectories. These results suggest that the movement trajectories we found in our earlier experiments do not originate from visual experience of the sighted subjects.


The reflex sensitivity was determined during various phases of rhythmic mandibular open-close movements. Subjects made such movements at their natural chewing rate controlled by a metronome. An external force, supplied by a solenoid in a magnetic field, act on the mandible in a downward direction during the closing phase, stimulating food resistance in the chewing phase. In this force a downward directed force-pulse (10 ms; 5 N) was randomly given at various phases of the open-close cycle. The jaw movement and force as well as the activity of masticator, temporal and digastric muscles were recorded. Only weak or no reflexes were observed during the opening phase. A mono-synaptic reflex was observed in the elevator muscles if the force-pulse was given during the closing phase of the chewing cycle. The ratio of the reflex activity and the background muscle activity was largest at the beginning of the closing phase. It may be concluded that the fusimotor system plays an important role in controlling muscle activity at the first engagement of the food between the teeth and that it is also involved in further load compensation during the closing phase.
central motor drive and ankle rotation did not account for observed H reflex inhibition during pedalling. W.E. McIlroy, D.F. Collins, P.J. Whelan, and J.D. Brooke, Human Biology, University of Guelph, Canada, N1G 2W1.

Human soleus H reflexes are depressed during walking, running and pedalling. What remains unclear are the sources of this inhibition. This study looked at two potential sources: 1) ankle rotation and 2) the central motor drive to move the limbs. These two factors were selected because in other paradigms they have been shown to strongly influence spinal reflex transmission, and they can be distinguished as peripheral and central influences, respectively.

Ankle Rotation: Subjects pedalled a cycle ergometer with and without a brace which prevented ankle movement. H reflexes sampled in the recovery phase during pedalling with the brace were not significantly different from those during normal pedalling. (Mean H reflexes: Normal 5.3% SD 4.8, With brace 9.4% SD 6.0 - expressed as percent of control H reflexes). H reflexes from both pedalling trials were significantly lower than control H reflexes during sitting.

Central Drive: Subjects remained relaxed, seated on a tandem cycle ergometer, while the limbs moved through the pedal cycle by a second cyclist. H reflexes sampled in the recovery phase of this passive movement were not different from those recorded during active pedalling. However, they were both significantly lower than the control reflexes (Mean H reflexes: Normal 6.9% SD 6.0, Passive 9.5% SD 11.0 - expressed as percent of control H reflexes).

The presence of substantial inhibition in the absence of that central motor drive may implicate peripheral inputs as an important source of inhibition. In support of this, we did not see a significant change in H reflex magnitude when ankle rotation was suppressed. It is possible that summed convergence from several peripheral sources is largely responsible for the inhibition of H reflexes during pedalling. (Supported by NSERC.)

reCiprocal (R) and Coactivation (C) Commands for WRIST MOVEMENTS, M.F. Levin, A.G. Feldman, J.E. Milner and Y. Lamarre, Rehabilitation Institute of Montreal, Canada H3S 2J4; CSIN & Institute of Biomedical Engineering, University of Montreal, Canada, H3C 3J7. According to the equilibrium-point hypothesis, movements are produced by means of displacement of the invariant torque/angle characteristic (IC) of the joint and change in the slope of the IC. Displacement is in the direction of the central R command while the C command specifies the slope of the IC. These commands were investigated in single joint wrist movements by perturbation methods. Subjects made free flexion movements to a target at 30° or movements against resistance presented opposing assistive loads. They were instructed not to correct the deflections arising in response to perturbations. Both peak velocity and EMG patterns were strongly affected by load conditions. Subjects underload or overloads the target with opposing assistive loads, respectively. However, after unloading (700 ms later), the target position was regained indicating that the IC was stable despite the perturbation. In two other experiments, subjects initially targeted a fixed target with opposing assistive loads, while the load was not presented in some trials. Depending on load conditions, the subject established different final IC positions. The slope of the IC was the same regardless of the magnitude of its shift. It is concluded that R and C commands are exerted independently. R and C commands are expressed via central commands (R and C).

determinants of jaw movement in mastication and speech. D.J. Otton, D.G. Feldman, J.R. Flanagan, and K.G. Munihall, McGill University, Montreal, Canada; Institute of Biomedical Engineering, University of Montreal, Montreal, Canada; Queen's University, Kingston, Canada.

We report recent X-ray microbeam studies on human jaw movement kinematics in mastication and speech. Empirical findings are compared to simulations of jaw movement based on the equilibrium point hypothesis (λ model). In speech trials, subjects produced consonant-vowel-consonant sequences at either normal or loud speech volumes. In the mastication trials, subjects chewed unilaterally on rubber tubes which varied in diameter and compliance. During mastication, we recorded the compression of the bolus. This was transformed into estimates of bite force using separately obtained functions relating applied force to tubing compression. Our previous work showed that jaw rotation and translation in speech can be separately controlled. For example, when rotation was plotted against translation, straight line paths were observed whose slopes depended on the consonant composition of the utterance. For a λ model, separate central commands to be defined for jaw rotation, jaw translation and co-activation of muscles without motion. Suppression of commands can predict observed combinations of jaw rotation, translation and bite force.
443.1

OPTIMAL STIMULATION OF PARASAGITTAL MOTOR CORTEX USING THE MAGNETIC COIL. Jean R. Cracco*, Nasser F. Hassani*, Paul S. Paul^, Mani Somasundaram^ and Vehe E. Amassian. SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203

We investigated the most effective method of exciting parasagittal motor cortex with a magnetic coil (MC) in 4 subjects. A round (9.4 cm diameter) MC which delivered an essentially monophasic pulse (first phase 3X larger than 2nd) was moved over right parasagittal cortex from various directions; CMAPs were recorded from contralateral foot and TMS was performed. The threshold was determined before and after the direction of current in the MC was reversed by a switch. The threshold for the left foot and responses was always lowest when the MC approached parasagittal cortex from the front and the current was counter-clockwise in direction on the MC, i.e. the induced current flowed clockwise in the cerebral cortex from right to left parasagittal cortex and then from left to right more anteriorly. Therefore, this location, orientation and current direction are optimal for excitation of projection neurons in right parasagittal cortex.

Defining the optimal position and orientation of the MC in excitation parasagittal motor cortex is of particular importance in the study of patients with spinal cord disease and in intraoperative monitoring.

443.2


To evaluate timing of reorganization of motor outputs in human motor cortex, we studied motor evoked potentials (MEP) to transcranial magnetic stimulation (TMS) in anesthetized monkeys immediately before, during and after unilateral anesthetic block of the distal arm below a pressure cuff inflated at the elbow. Anesthetic block was maintained for 70 and 45 minutes in the right and left arm respectively. Electromyographic recordings were made from muscles immediately proximal (biceps, deltoid) and distant (abductor pollicis brevis) to the pressure cuff and in 1 subject, contralateral forearm and hand muscles.

With anesthesia, the MEP in abductor pollicis brevis became gradually smaller and eventually disappeared. Over the same time course the MEP became progressively larger in biceps (p<0.0001), but was unaffected in deltoid. In the subject studied, MEP amplitudes from muscles contralateral to the deafferented limb were unchanged.

Reversal of the changes were observed within minutes of discontinuation of anesthesia. The rapid time course of reorganization of motor outputs, by which a certain region of motor cortex can reversibly influence a new set of muscle, provided further evidence for preexisting connectivity, which may underlie neuronal plasticity.

443.3


In order to examine the hypothesis that different cortical motor areas are involved in the generation of either self-paced or externally-triggered limb movements, we used H2O 15O positron emission tomography to record regional cerebral blood flow (rCBF) in 10 subjects who performed unilateral finger flexion tasks while fixating a visual target. We studied three different tasks: Movement execution (ME), in the self-paced (SP) condition, the subjects moved a joystick handle by finger flexion once every 2-3 seconds. Each movement caused the fixation target to briefly flash. In the visually-triggered (VT) condition, subjects were instructed to move in response to the target flash. In the control condition the target was flashed at the same rate: the subjects were instructed to maintain fixation but not respond. Movement onset times were recorded in the SP condition and then used to pace the subsequent two conditions. Difference images representing net rCBF changes were obtained by task pair subtraction. Response localization was determined by 3D registration of each subject's blood flow and MRI. A repeated measures ANOVA model was used for statistical analysis of rCBF changes.

We found equal rCBF increases in the SP and VT tasks in the precentral and lateral superior frontal gyri (p<0.01). Responses were more strongly lateralized in the precentral gyrus. In contrast, the contralateral medial superior frontal gyrus (corresponding to the supplementary motor area) was preferentially activated during the SP movements (p<0.01). These results provide further evidence for regional cortical specialization for control of kinetically similar limb movements that differ markedly in their behavioral context.

443.4

PRIMATE FRONTAL CORTEX: VISUOSPATIAL VS. VISUOMOTOR ACTIVITY S.P. Wise and G. di Pellegrino. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

We examined neuronal activity in the premotor (PM) and prefrontal (PF) cortex while a rhesus monkey performed a visuomotor, spatial matching task. The monkey fixated and held a manipulandum beneath a central light emitting diode (LED), which had 8 LEDs arranged in a circle around it. On each trial, 1 of the 8 LEDs illuminated for 0.5 s (the stimulus plus, PF, or the other LEDs illuminated sequentially for 0.1 s at delays of 0.55 or 0.75 s. At the end of this delay period, the PS illuminated again for 0.1 s whereupon the monkey had to execute a forelimb movement within 650 ms (or could break fixation). There were 2 response conditions: the monkey moved the manipulandum (1) to a predetermined target regardless of PS location (the incompatible condition) or (2) to the PS location (the compatible condition).

Because the visuospatial stimuli were physically and retinotopically identical in the two conditions, and because the monkey's arm was immobile in the incompatible condition, the PS location identically, the behavioral design allowed us to test the hypothesis that task-related activity simply reflects a sensory response. The alternative is that the motor instructional significance of the stimuli affects the cell activity. Second, because in the incompatible condition a single motor response followed a variety of visuospatial cues, we could examine whether the activity reflects simply motor aspects of the animal's behavior. The alternative is that some aspect of the visuospatial cue affects cell activity. Our results from 94 PM cells show that for many cells both behavioral hypotheses can be rejected. Accordingly, PM activity, regardless of any temporal correlation with either the sensory signals or the onset of movement, reflects integrative aspects of the visuomotor behavior, i.e., aspects of behavior that cannot be simply characterized as either sensory or motor in nature. A sample of 55 PF cells suggests a higher proportion of cells there simply reflect the sensory, possibly visuospatial, aspects of the stimuli.

443.5

SPECIFIC EFFECT OF MOTOR LEARNING ON SET-RELATED CELLS OF THE PREMOTOR CORTEX IN PRIMATE. L. Germain, Y. Lamare, M.T. Parent. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada H3C 3J7.

In order to assess the effect of learning on set-related cells in the precentral and cingulate cortex, we trained one primate to perform elbow flexion (F) or extension (E) according to a two choice instructed delay paradigm. During training (A) the presentation of the auditory instruction (400 Hz tone for 5 or 1000 Hz tone for F) was not randomized, and the performance was below chance level (50%). After training (B) the presentation of the auditory instruction was randomized and the performance was 90% correct. To assess the time course of learning, we tested the animal in a 500 msec control period following a stimulus by a 400 msec auditory instruction then by a randomized immobile waiting period of 500 to 1500 msec terminated by a small kinesthetic go-stimulus. A total of 386 movement related cells were recorded and 267 set-related cells were found in this sample. A specific and significant increase of the proportion of set-related cells was observed in the premotor cortex from A) 20% (±SE 3.5%) to B) 65.6% (95%) (x2 = 33.5, p<0.005) furthermore the proportion of directionally set-related cells increased in this area from A) 11/22 (50%) to B) 54/65 (83%) (x2 = 9.4, p<0.005). The mean coefficient of directionality (value between F and X) of set-related cells increased specifically and significantly from A) 3.7 ± 1 to B) 7.6 ± 4 (t = 3.19, p = 63, x2 = 0.005) in the premotor cortex. The proportion of cells with auditory responses increased from A) 145 (0.25%) to B) 1565 (22%) in the premotor cortex and also from A) 730 (23%) to B) 13/30 (43%) in the dentate nucleus. These data suggest a predominant effect of learning an arbitrary association between an auditory instruction and a movement on a set-related cell in the precentral cortex. (Supported by MRC of Canada grant in Neurological Sciences).

443.6


The functional organization of layer II-III pyramidal neurons in area 4 of cat motor cortex were examined after intracortical injections of biocytin. All of the cells examined produced long-term potentiation (LTP) in response to tetanic stimulation of the somatosensory cortex. Their primary axon, originating from the soma or dendrites, emitted intracortical axon collaterals before entering the subcortical white matter. The axon collaterals had both vertical and horizontal patterns of termination. Collateral branches originated in the immediate vicinity of their parent soma and dendrites. In addition, most neurons had axon collateral branches that projected horizontally for long distances. These long range, horizontal branches projected to and terminated within the same layer their parent soma was located, and ranged in length from 1 to 4 mm. Relatively shorter horizontal branches were emitted from the primary axon in deep layer V and layer VI. Within the superficial layers, the long collaterals typically formed clusters of terminal fields, having a diameter of the order of 1 mm. The spatial arrangements of these intracortical connections indicate that these neurons are involved in ‘clustered’ networks of excitatory connections within the motor cortex. Specifically, these findings suggest that axonal collaterals in the superficial layers of the motor cortex would affect the firing patterns of cells located in spatially segregated cortical columns. Supported by NSF grant #9021162 (A.K.) and NIH grant # NS-10705 (H.A.)

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443.7 COULD THE CORPUS CALLOSUM PREVENT INTERHEMISPHERIC TRANSFER OF LEARNING TO ENABLE HEMISPHERIC SPECIALIZATION IN A COGNITIVE TASK? THE ROLE OF THE PRIMATE CORPUS CALLOSUM IN NORMAL AND SPLIT-BRAIN RHESUS MONKEYS. B. Prelovški, R. J. Engel, W. Reger, W. Polt, Weissenau-Field-Station, Tübingen University, Rasthalde 3, D-74007 Tübingen, FRG.

In a series of experiments the development of distal and proximal sensory-motor skills and their intermanual transfer was studied in split-brain and intact rhesus monkeys. When the monkeys had to learn to produce a specific force between the fingers of one hand, no transfer of learning to the opposite hand was observed in intact animals, in a comparable task involving force production, arm movements delayed transfer was observed. Delayed transfer was also found in a callosotomized animal postoperatively trained and tested for transfer in the force production task. Furthermore, when animals were alternating between hands during training on the finger task, performance with both hands tended to become equalized after callosotomy.

The results, so far, suggest that the corpus callosum has variable functions in sensory-motor control. Among others, it may modulate bilateral sensory-motor interactions taking place bilaterally, and, in prolonged unilateral training, it may inhibit interhemispheric transfer allowing for independent skill development and performance with either extremity. If confirmed, this would carry important implications for a possible theory of handedness and laterality as well as bilateral motor functions. (Supported by the German Research Foundation.)

443.9 DEPTHS OF VISUAL REACHING BEHAVIOR BY LOCAL INJECTIONS OF GABA	extsubscript{A} AGONIST AND ANTAGONIST (BACLOFEN AND PHACLOFEN) INTO THE VENTRAL ASPECT OF THE PRIMATE CORPUS CALLOSUM. K. Kawanita and K. Kubota, Dept. of Neurophysiology, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

The role of intracortical GABA	extsubscript{A}ergic inhibition in the performance of Visual Reaching was studied in two monkeys (Macaca mulatta). Baclofen (2.5 μg/pl) or Phaclofen (0.2-1 μg/pl) was injected locally into the hand motor area (MC) or the post-arcuate hand premotor area (PM), while monkeys were performing the task by hand. When Baclofen was injected into the MC (10 sites), neuronal bursts and convolutions of forearm muscles appeared gradually. Numbers of the task performance decreased gradually with decreased current. But when Phaclofen released his hand slowly from hold key to reach target key and pressed the target key for longer periods, Number decrease was due to decreased efficiency of the task. The results suggested that monkeys tended to press the target key. Both reaction and movement time were prolonged. Similar effects, though less pronounced, were also observed in intact animals; in a comparable task involving force production, arm movements delayed transfer was observed. Delayed transfer was also found in a callosotomized animal postoperatively trained and tested for transfer in the force production task. Furthermore, when animals were alternating between hands during training on the finger task, performance with both hands tended to become equalized after callosotomy.

The results, so far, suggest that the corpus callosum has variable functions in sensory-motor control. Among others, it may modulate bilateral sensory-motor interactions taking place bilaterally, and, in prolonged unilateral training, it may inhibit interhemispheric transfer allowing for independent skill development and performance with either extremity. If confirmed, this would carry important implications for a possible theory of handedness and laterality as well as bilateral motor functions. (Supported by the German Research Foundation.)

444.3 EFFECTS OF MUSCIMOL INJECTION INTO THE DORSAL AND VENTRAL ASPECTS OF THE PREMOTOR CORTEX IN MONKEYS PERFORMING A CONDITIONAL MOTIVE TASK. K. Kurata, D. S. Hoffman, and J. Kawamoto. Department of Physiology, Tohoku University School of Medicine, Sendai, 980, Japan, and Research Service (151), VA Medical Center, 41000 NYC, NY 10041.

Muscimol (Sigma, 5μg/ml solution) was injected (1.3μl) into the dorsal and ventral aspects of the premotor cortex (PM) of two Japanese monkeys (Macaca fuscata) while they were performing a motor task (spread finger extension or flexion to a target). The correct movement was instructed by either: (1) conditional color cues (green or red LED) equidistant from the targets or (2) conditional direction cues (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus at (near) threshold for flexion (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus at (near) threshold for flexion (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus at (near) threshold for flexion (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus at (near) threshold for flexion (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus at (near) threshold for flexion (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus at (near) threshold for flexion (left or right LED). When the green or red LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required.

444.10 PERSISTENT DEFICITS OF EYE MOVEMENTS, GOAL-DIRECTED ARM MOVEMENTS AND VISUO-SPATIAL ORIENTATION FOLLOWING SMALL UNILATERAL PMd, PMv OR VENTRAL ASPECTS OF THE PRIMATE CORPUS CALLOSUM LESIONS. W. Held, H. Wild, D. Peterzen. Dept. of Neurology and Neuroanatomy, University of Tübingen, W-7400 Tübingen, FRG (SPON: European Brain and Behaviour Society).

Various visuo-spatial, visuo-vestibular and visuo-motor functions have been ascribed to the posterior parietal cortex. In a lesion study, we tried to correlate deficits of these functions with each other and with the cortical areas involved in 8 patients (5 left- and 3 right-hemispheric) with small chronic unilateral lesions around the intraparietal sulcus, according to MR imaging, compared to 10 normal controls. The assessment of visually and vestibularly induced eye movements and psychophysical assessment of the subjective straight ahead (SSA), the subjective visual vertical (SVV) and the latencies of vection under optokinetic full-field stimulation, fast goal-directed arm movements were recorded by driving a pen to visual targets on a digitizing tablet.

Removes showed that the patient's acute symptoms (visual hemineglect, spatial disorientation, optic ataxia, constructive apraxia, deviation of SSA) had almost disappeared. 3 of them, however, had a marked contralateral tilt of their SVV (>20°), associated with impaired circular vection, asymmetries of optokinetic and vestibular nystagmus (reduced gain of ipsilaterally directed slow phases) and an ipsilateral deviation in line bisection test. 3 others had a contralateral bias in the optokinetic nystagmus test, furthermore a spontaneous nystagmus (SVN; slow phases deviating ipsilaterally) and - only 1 of them - a direction-specific deviation of goal-directed hand movements (ipsilaterally) and of the SSA (contralaterally). We assume that the multidimensional bias found in some of the cases might reflect a distortion of egocentric localization. Others have compensated this bias, maybe by means of an SPN.
Gaussian distributions, reflecting spontaneous synaptic inputs originating from decay time constants averaged 3.6 ± 1.5 ms; in 34 SMCs, rise times averaged 1.0 ± 

High frequency, large sEPSCs were not abolished by whereas SMCs were defined as those with APs large afterhyperpolarizations, whereas SMCs were spiny and had thorny excrescences on their proximal dendrites.

whole-cell patch-clamp technique in young rat hippocampal slices. AHIs were modifications during neural activity may influence hippocampal circuitry and thus onsets of approximate 3 sec. The pattern of the stimulus-evoked changes in optical properties was correlated with maps of the intensities of evoked field potentials in layers 1-3 of the various cortices. Intrinsic signals were recorded at wavelengths from 500 to 750 nm but the largest signals were observed at 550 and 600 nm which may be due to light absorption by cytochrome oxidase a and c (550 nm: peak signal Δ Re = 1.91%; noise = 0.15%). LEC stimulation occasionally evoked seizure activity in the LEC. A large change in the intrinsic signal was found to spread across the medial entorhinal cortex/subiculum to the amygdala and pyriform cortex across the recorded seizure. Therefore, it is possible to image intrinsic optical signals in the guinea-pig isolated whole brain preparation. This technique may be useful for the study of functional activity patterns and the spread of seizure activity. Supported by MRC (Canada).

**POSTSYNAPTIC MECHANISMS IN NEUROTTRANSMISSION**

**444.1**

PHOTOLYTIC MANIPULATION OF [Ca²⁺] YEARS CONTROLS HYPERPOLARIZATIONS IN HIPPOCAMPAL PYRAMIDAL CELLS.

Experiments were performed to assess the reason for the slowness of the AHP in rat CA1 pyramidal cells. This Ca²⁺-dependent K current reaches a peak 500 ms following Ca²⁺ entry during spikes (BG). In order to raise Ca²⁺ instantaneously throughout the cell, conventional intracellular recordings were made with electrodes containing DM-nitrophen loaded with 30% Ca²⁺-UV light photolyzes DM-NITROPHEN with an attendant reduction in Ca²⁺ affinity, thus releasing Ca²⁺. For photolysis, a UV flashlamp (flash duration 1.6 ms) was focused onto the slice surface. A single flash could evoke a 10 mV hyperpolarization from rest (-70 mV). The onset of 30 to 50 ms was limited by the membrane time constant. Durations of initial responses were 20-60 s. DM-nitrophen filled cells displayed small AHPs presumably as a result of Ca²⁺ buffering; these reduced AHPs showed the same slow kinetics as control responses. Flash responses and AHPs showed a common sensitivity to 1 μM tetrodotoxin, suggesting that both represent activation of the same current. When electrodes contained the caged BAPTA derivative Diox-4, robust AHPs were observed. A flash (now to release the Ca²⁺ buffer) at the peak of an AHP curtailed the response immediately. Subsequently, slow AHPs could not be evoked. These results show that rapid manipulations of [Ca²⁺] can cause similar rapid changes in AHP current. By contrast, this implies that spike induced Ca²⁺ entry causes slow [Ca²⁺] changes over the majority of AHP channels, possibly this is a consequence of spatial localisation. Supported by NIH grant NS 15114.

**444.2**

HYPERPOLARIZING AND DEPOLARIZING GABA RECEPTOR-MEDIATED DENDRITIC INHIBITION IN AREA CA1 OF RAT HIPPOCAMPUS.


Supported by MRC (Canada).

**444.4**

Potentialation of Ca²⁺ changes in hippocampal CA3 pyramidal neurons by modulatory neurotransmitters. J.A. Connor, W. Miller and J. Petrozza.

Supported by MRC (Canada).

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**444.3**

SLOWER SPONTANEOUS EXCITATORY SYNAPTIC CURRENTS IN SPINY VS. ASPINY HIPPOCAMPAL HILAR NEURONS.

Cortex III

Supported by NSF grant. Supported by NIH grant NS 28135.
44.5 GLUTAMATE RECEPTOR DESSENSITIZATION GOVERNS THE STRENGTH OF EXCITATORY SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. I.J. Thompson, A.J. Nicoll, Dept. of Physiology and Pharmacology, University of California, San Francisco, CA 94143. 

Rapid perfusion experiments with the non-NMDA type of glutamate receptor inhibitor, dizocilpine methanesulphonate, and the NMDA receptor inhibitor, magnesium, show that the strongly Ca-dependent form of synaptic transmission in the hippocampus is governed by a Ca-dependent desensitization of the NMDA receptor. Desensitization is apparent within 1 sec of the initial agonist application and is characterized by: (i) a rundown of the Ca current until no further rundown was observed, (ii) a reduction in the Ca conductance, which was still measurable 30 sec after the initial agonist application, and (iii) a decrease in the agonist concentration required to produce a half-maximal increase in Ca conductance. These findings indicate that the non-NMDA and NMDA receptors mediate the synaptic transmission in the hippocampus.

44.6 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS OF A GABA-MEDIATED INHIBITORY SEQUENCE IN THE HIPPOCAMPUS. Avoli M, Tascredi V, Siniscalchi A and Zona G. McGill University, Canada H3A 2B4; Università di Roma Tor Vergata, Rome, Italy.

Intracellular recordings with K-acetate-filled electrodes were made in slices of the adult rat hippocampus to study the orthodromic inhibitory sequence generated by CA1 pyramidal cells. In 43 of 72 cells studied, stimuli that were delivered in the stratum oriens generated an initial EPSP (ii) an early hyperpolarizing IPSP (peak latency-approx. 20 ms) (iii) an intermediate depolarizing component (peak latency-60-100 ms) (iv) a late, long-lasting hyperpolarizing IPSP (peak latency-120-160 ms; duration >400 ms). In the remaining cells this inhibitory response lacked the intermediate depolarizing component. The depolarizing component represented an active, GABA-mediated inhibitory post-synaptic potential (IPSP) which was selectively blocked by local applications of bicuculline or picrotxin on the apical dendrites (ii) it was enhanced by lowering the temperature or by the GABA-uptake blocker nitenpyram. Application of the CI-pump blocker furosemide reduced and eventually blocked both early IPSP and depolarizing component. These data demonstrate that under physiological conditions rat hippocampal pyramidal cells generate a depolarizing component that is presumably caused by an outward-directed Cl movement due to the activation of GABA receptors located on the apical dendrites. This novel mechanism might modulate hippocampal excitability under both physiological and pathophysiological conditions.

44.7 INTERACTION BETWEEN CHOLINERGIC AND HISTAMINERGIC CALCIUM SIGNALS IN MOUSE NEUROBLASTOMA CELLS. S.-H. Wang and S. Thompson.

Cholinergic and histaminergic cells in NIE-115 neuroblastoma cells show a phasic (PI) hydrolysis of calcium and calcium mobilization. Using fura-2 calcium imaging we find that carbachol (CBC) and histamine (HIS) evoke similar Ca "fingerprint" in the same cell, as defined by kinetics and spatial patterns of release from stores. The response to CBC is abolished by phorbol ester, implicating protein kinase C (PKC) in desensitization.

We examined the effects of long exposure to CBC and HIS. A Ca response was defined as <-50% change in fluorescence after 30 s exposure. Analysis was restricted to cells that respond to both agonists. A 5-minute exposure to 1 mM CBC desensitizes the cells by 37% to a second application of CBC, but this did not affect their responsiveness to HIS. Conversely, a 10-minute exposure to 10 μM HIS desensitizes the cells by 41% to a second application of HIS, while preserving the CBC response.

Desensitization is one way that receptor activation can alter synaptic efficacy. The lack of cross-desensitization here indicates that it may take place at PI hydrolysis which is not blocked by PKC-dependent mechanism.
445.1 IDENTIFICATION OF A DISTINCT POPULATION OF NERVE GROWTH FACTOR LIKE-IMMUNOREACTIVE CELLS WITHIN THE DORSAL HYPOTHYMYXUS E.S. Purcell, R.A. Murphy, Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada.

We have described a developmentally increased monoaminergic innervation of thymus in the spontaneously hypertensive rat (SHR), an animal model with a T cell dysfunction (Soc. Neurosci. 16(21):1200). We hypothesized that trophic factors induced this hypervascularization. To test this hypothesis, we examined NGF and NGF receptor immunoreactivity (IR) using indirect immunofluorescence. A well defined population of cells with a rounded morphology and strong NGF-IR was identified in the interlobular septae of the developing thymus. These cells were loosely associated with the vascular along which the innervation initially develops. The SHR thymus had an increased number of these cells which were more intensely labelled as compared to those seen in the Wistar Kyoto rat. Labeling for NGF receptor-IR was minimal, suggesting that the NGF-IR represents cellular production rather than receptor mediated uptake. More diffusely labeled cells were found in thymic cortex at older time points. In conclusion, a specific population of cells is immunoactive for NGF in the developing thymus. Their location, number and temporal appearance correlate with the development of thymic hypervascularization in the SHR. Supported by PHS grant MH44511.


The NGF proto-oncogene product (p140Protok) binds NGF and undergoes rapid autophosphorylation in response to this neurotrophic factor. EMS mutagenized PC12 cell lines deficient in high affinity NGF binding and unresponsive to NGF (PC12mrn; Green et al., J. Cell. Biol. 93:843-850, 1986) were used to determine if p140Protok is involved in transmitting a functional signal. Northern blot analysis using a p140-specific probe revealed that PC12mrn lines express significantly less NGF mRNA do wild type PC12 cells. Expression vectors encoding full length p140Protok were transiently transfected into NGF-IR cells by the lipofectin method. After 4 days of exposure to NGF, approximately 10% of the cells displayed long neurites ending in growth cones; without NGF, no neurites were observed. Control cultures either mock transfected or transfected with a plasmid lacking p140Protok did not grow neurites when exposed to NGF.

Treatment of the cultures with 200 nM K252a, a drug that specifically blocks NGF responses, effectively suppressed NGF induction of neurite outgrowth. NGF is not induced c-fos gene. These data indicate that NGF gene expression may be mediated through multiple signal pathways.

445.5 THE SCHWANN CELL-DERIVED TRUNCATED FORM OF THE NGF RECEPTOR ARISES FROM POST-TRANSLATIONAL PROCESSING A. Barker, F.D. Miller, T.H. Large, and R.A. Murphy Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada.

Low affinity NGF receptors have been identified on neurons in the peripheral and central nervous system and on several non-neuronal cell types. The putative tyrosine kinase receptor is present in the bound and soluble truncated form. We have undertaken to determine whether the truncated form is generated as a result of post translational proteolytic processing of intact membrane-bound receptor or from an alternatively spliced mRNA lacking a transmembrane domain.

Pulsed chase analysis of primary rat Schwann cells coupled with immunoprecipitations using antibodies to the intracellular and extracellular domains of the receptor were used to monitor receptor production. Three forms of the NGF receptor (Mr 80, 82, 85 kd) displaying a processing relationship were detected over a two hour chase period in cell extracts with only the 85 kd species appearing on the cell surface. Truncated receptor (Mr 50 and 52 kd) was detected in conditioned medium five hours after labelling but not intracellularly. PCR and RNase protection analyses detected no splice variants that could generate receptor and media conditioned by fibroblasts transfected with rat receptor cDNA, in which splicing cannot occur, produced truncated receptor as well. Therefore, the truncated form of the receptor arises from post translational processing of the intact receptor rather than as a distinct translation product.

445.6 NGF REGULATES NEURONAL GENE EXPRESSION IN A CONCENTRATION DEPENDENT FASHION. Y. Ma, R.B. Campenot, and F.D. Miller. Dept. of Anat. and Cell Biol., Univ. of Alberta, Edmonton, Alberta, CANADA.

Developing sympathetic neurons respond to systemic administration of NGF by alterations in gene expression (Mathew and Miller, Dev. Biol., 141, 84, 1990; Millar et al., JCB, 112, 303, 1991). To determine the direct effects of different concentrations of NGF on sympathetic neurons, we studied the regulation of mRNAs encoding the low affinity NGF receptor (NGFR), Tau a-tubulin (Tat), and tyrosine hydroxylase (TH) in cultured rat sympathetic neurons at the postnatal day 1 SCG. Northern blot analysis of total cytoplasmic RNA isolated from sympathetic neurons cultured for 5 days in 200 ng/ml 2.5S NGF revealed that, as the NGF concentration increased, neurons expressed correspondingly increased levels of three mRNAs. Both LNGFR and TH mRNAs were increased at 7-fold, and Tau mRNAs 4-fold in neurons cultured in 200 ng/ml versus 10 ng/ml NGF. Furthermore, when neurons were cultured in 10 ng/ml 2.5S NGF for 5 days, and the concentration was subsequently increased to 200 ng/ml LNGFR, TH, and Tau mRNAs all increased within 2 hours. LNGFR and TH mRNA levels continued to increase for 24 hours following the NGF increase (last time point examined) while Tau mRNA reached a maximum level 4 hours after NGF increase. These experiments show that NGF directly modulates the expression of these neuronal genes in a concentration-dependent fashion. Since tubulin and tyrosine hydroxylase are enzymes of the axon and nerve terminal the NGF concentration dependency of their genes likely reflects a step in the regulation of neuronal growth by NGF. These data also raise the possibility that the effects of NGF gene expression may be mediated through multiple signal transduction pathways that are activated at different concentrations.
INDUCTION OF NGF OCCURS BY MULTIPLE MECHANISMS.


NGF has been shown to be induced in various systems by seemingly unrelated compounds, including the NGF precursor, cell surface NGF receptors, and multiple agents which block NGF synthesis or release. Here we present evidence that the NGF signal transduction cascade which may mediate induction of NGF by various compounds. It was found that NGF, as measured by ELISA, could be induced, although to varying extents, in mouse L cells using a variety of the above types of compounds. Further, serum and the cell surface derivative, 4-methyl catechol (MC-4)-MC, appeared to induce NGF synergistically. Northern blot analyses of NGF mRNA suggested that the induction of NGF by both MC-4 and serum, as well as their synergistic induction, are reflected at the transcriptional level. MC-4 and the beta receptor agonist, agonist, also appear to induce NGF by different means: the beta receptor agonist propranolol blocked induction by isoproterenol, but not by MC-4. The molecular basis of these findings is now being investigated, including studies to analyze the DNA sequence elements within the NGF promoter and the proteins that bind to these elements that are required for both basal and induced levels of transcription.

C-fos transcription is also induced by a number of other agonists, including isoproterenol, also appear to induce NGF by different means: the beta receptor agonist propranolol blocked induction by isoproterenol, but not by MC-4. The molecular basis of these findings is now being investigated, including studies to analyze the DNA sequence elements within the NGF promoter and the proteins that bind to these elements that are required for both basal and induced levels of transcription.

Comparisons of NGF receptor distribution on PC12 and Schwann cells using antibodies 217c and 192-IgG.

F. Ferrari, M. Fabrizi, A. Brunst*, L. Potenziani, and P.E. Spoerri.

Fidia Research Labs., Abano T. (PD), Italy, 1 Dept. of Pharmacology, Univ. of Padova, Italy

Recently, we reported that the antibody 217c recognizes a different epitope on the NGF receptor (NGFR) than does 192-IgG (Ferrari et al. Exp. Neurol., 1991). These studies were extended by showing that binding of 217c and 192-IgG recognize epitopes located on the extracellular domain of the low-affinity NGF receptor (LNGFR) as well as the LNGFR, but not the high-affinity (HNGFR) cross-linked complex (Meakin et al., J. Neurosci., 1991). We have employed immunoelectron microscopy to examine the topographical distribution of antigens recognized by 217c and 192-IgG on NGF-primed PC12 cells (which have both LNGFR and HNGFR) and Schwann cells (which have only LNGFR). The two antibodies exhibited similar immunogold reactivity. However, in NGF-primed PC12 cells, colloidal gold was observed at regular intervals along perikaryal and neuritic surfaces. Prominent immunoreactivity was frequently localized in coated endocytotic vesicles, on SER and in secondary multivesicular lysosomes. In contrast, immunoreactivity in Schwann cells was only present along the plasma membrane. We conclude that these antibodies may be useful to follow the fate of the two forms of NGF.
**445.13**


A nuclear protein designated as 52kDa slow migrating protein (52kDa SMP) was found to show a high-affinity binding to antibodies raised against the C-terminal sequence (DG1V1G2DVAHVRKHH,GR, has considerable homology to the C-terminal sequences of several proteins such as myosin, desmin, and myoglobin.) Receptor-mediated tyrosine phosphorylation of the nuclear protein was investigated. Cells were treated with different concentrations of NGF and eventually by 1/2 min and then assayed for the phosphorylation of the nuclear protein. The results indicate that the phosphorylation of the nuclear protein is time- and concentration-dependent.

**445.15**

**DISSOCIATION OF CHANGES IN GTP CYCLOHYDROLASE, TETRAHYDROBIOTERPINE AND TYROSINE HYDROXYLASE INDUCED BY NGF IN SUPERIOR CERVICAL GANGLIA.** M. Ishihara and G. Kapatos. Department of Psychiatry, Wayne State Univ., Detroit, Ml 48235

GTP cyclohydrolase (GTPCH) is the first and rate-limiting enzyme in the tetrahydrobiopterin (BH4) biosynthetic pathway. BH4 is known to serve as a specific cofactor for tyrosine hydroxylase (TH). NGF induces an increase in TH activity and in BH4 biosynthesis, which is not blocked by inhibitors of PH, PKA, or PKN. This study was designed to investigate the kinetics of TH and BH4 induction by NGF and to determine if the increase in TH activity is a consequence of an increase in BH4 biosynthesis.

**445.16**

**PHOSPHORYLATION AND ACTIVATION OF SER/THR KINASE OF B-RAF BY NERVE GROWTH FACTOR IN PC12 CELLS.** M. Oshima*, U. R. Rapp*, and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, Bethesda, MD 20892, and Laboratory of Neurosciences, National Cancer Institute, Frederick Cancer Center, Frederick, MD 21701

The ras family of proto-oncogenes is a group of proteins that are involved in regulating the growth and proliferation of cells. One of the members of this family is B-raf, which is known to be activated by nerve growth factor (NGF) and to play a role in the regulation of the growth of sympathetic neurons. This study was designed to investigate the mechanism by which B-raf is activated by NGF and to determine if the activation is dependent on the phosphorylation of the protein.

**445.17**


Increased hydrolysis of phosphatidylinositol (P) occurs as a consequence of NGF to PC12 cells in culture. Receptor-mediated, NGF-stimulated PI turnover is prevented by treatment with the PKA-dependent activity of PLC. Treatment of PC12 cells with PI for 5 min with NGF leads to a 2-3-fold increase in the phosphorylation of PLC-Y. Removal of extracellular Ca²⁺ blocks the action of NGF. Phosphoamino acid analysis indicates an increase in both phosphoserine and phosphothreonine. Time-course studies show that the phosphorylation of PI occurs within 30 sec following NGF addition, and that it is more rapid than the PI-mediated increase. Pretreatment with the PI kinase inhibitor genistein (150 μM) blocks both NGF-induced PI turnover and PI-mediated PI phosphorylation. These data suggest that NGF-stimulated PI hydrolysis occurs in increased tyrosine phosphorylation. The study is supported by grants from the NIH and March of Dimes.

**445.18**

**NERVE GROWTH FACTOR (NGF)-STIMULATED RELEASE OF ADRENERGIC NEUROTTRANSMITTERS AND UPTAKE OF CALCIUM BY PC12 CELLS.** M. Oshima*, U. R. Rapp*, and G. Guroff. Section on Growth Factors, NICHD, NIH and Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892.

It has been shown that NGF stimulates a calcium-dependent release of catecholamines from PC12 cells. In addition, NGF also stimulates the uptake of calcium into cells attached to collagen-

polylysine coated plates by 60-80% within 3 min, but this effect is transient, lasting for 5-10 min. The effect of NGF was blocked by inhibitors of N-, T-, and L-type calcium channels, and by antagonists of calcium.

**445.19**

**PARTIAL CHARACTERIZATION OF NERVE GROWTH FACTOR V THURSDAY AM**

**445.20**

**ACTIVATION OF THE CATALYTIC SUBUNIT OF CALCITRIGGERED PROTEIN KINASE BY PROTEIN KINASE N (PKN).** C. Volonte and I.A. Greenfield Department of Pathology, Cornell University Medical College, Physicians and Surgeons, New York, N. Y., 10032

Protein kinase N (PKN), a serine protein kinase that is rapidly activated by NGF and other agents in PC12 cells and other cell lines, increases the phosphorylation of certain substrates. It is known that PKN is activated by NGF, but not by PKA and several additional substrates. These data suggest that PKN may activate PKA by promoting a conformational change that is more enzymatically active. PKN and PKA appear to be widely expressed, and past studies have shown that PKN can be activated in cells via a PKA-dependent pathway. The occurrence of PKN/PKA interactions within cells thus would have interesting biological implications for signal transduction. Supported by grants from NIH and March of Dimes.
ACTIVATION AND DISTRIBUTION OF PROTEIN KINASE C (PKC) ISOYMEs IN PC12 CELLS AFTER TREATMENT WITH PKC STIMULATORS. J. J. Morris, R. K. Singh, A. P. Fields, and K. L. Ness. Depts. of Biochemistry and Pharmacology, Case Western Reserve University, Cleveland, OH, and Dept. of Biological Chemistry, The Chicago Medical School, N. Chicago, IL. PKC is a multifunctional nerve growth factor family, phospholipases (PM), and the macroscopic lactate brenostat (BR) but is not required for the generation of neurites in PC12 cells. We have characterized the response of PKC isoyme to various agents using isotype specific antibodies directed against peptide determinants of PKC γ, β1, and γ. Western blots have shown that all three isoymes are present in significant amounts in the cytosol of control cells with γ the most abundant. After acute treatment with NGF, PKC κ and β1 levels remain unchanged but immunoreactive PKC γ-Ymphily disappear from all cellular fractions and then recover by 1-2 h. We are currently investigating the mechanism of this loss. Chronic down regulation by PMA or BRY leads to rapid loss of PKC κ and β1 but PKC γ persists even at 24 h. Activity measurements have shown that the cytosolic PKC activity increases significantly from control values. High dose CNTF significantly decreases significantly from control values. This data suggests that CNTF might play a trophic role early in C NSF development. We have used the NLT model as a way to manipulate the effects of endogenous opioids on CNS development in vivo. Since nerve growth factor (NGF) is involved in CNS development, NGF was assayed in 3 brain regions after 1 or 2 vks of NLT. NLT led to a decrease of NGF content, measured by a 2-site ELISA, in striatum (ST) and HC, with no effect on CB NGF. In contrast, astrocyte cultures from CB or ST of NLT-treated rats contained increased levels of NGF, while HC astrocytes were unchanged. Opiate receptor binding assays are underway to determine whether NLT acts directly on astrocytes or indirectly via another mediator.

GROWTH FACTORS AND TROPHIC AGENTS V

CILIARY NEUROTROPHIC FACTOR (CNTF) EFFECTS ON RAT SPINAL CORD NEURONS IN VITRO. SURVIVAL AND EXPRESSION OF CHOLINE ACETYLTRANSFERASE AND LOW AFFINITY NGF RECEPTORS. P. Mesulam, P. Buhmann, S. Vang, Dept. Biol. Univ. Cali. San Diego, La Jolla, CA. CNTF promotes the survival of a variety of neuronal cell types, induces cholinergic properties in sympathetic neurons and differentiation of O-2A progenitor cells into type-2 oligodendrocytes. It has been found that the effects of CNTF and NGF on E14 rat spinal cord cultures. After 7 days of treatments with CNTF (human recombinant or purified from rat sciatic nerve; 100 TiM) an increase was seen in: i) the number of neurons not stained with cholacyltransferase (CHAT) or low affinity nerve growth factor receptor (LNGFR) antibodies (identified by immunostaining for neuron-specific enolase); ii) the number of α-motoneurons (0.5% of the total neuronal population), identified by their size (>25 μm), morphology and CHAT and LNGFR-immunoreactivity, and iii) a population of small to medium sized (5-25 μm), CHAT- and LNGFR-positive neurons, representing 5-10% of the total neuronal population. Delayed administration of CNTF revealed that the CHAT-NGFR-negative neurons and α-motoneurons were dependent on CNTF for their survival. In contrast, the small CHAT-NGFR-positive neurons were not dependent on CNTF for survival, but were induced by CNTF to express these two markers. These observations suggest that CNTF is a neurotrophic factor for α-motoneurons and a large unidentified population of spinal cord neurons, as well as a regulator of CHAT and LNGFR expression for some neurons of the fetal spinal cord. This latter regulatory role was also observed for a small number of neurons cultured from various other brain areas. Supported by NINDS grants NS16349 and NS27047.
TROPHIC ACTIONS OF BDNF ON DOPAMINERGIC NEURONS IN VITRO: J. Pharmacol. Exp. Ther. 248:842) was used to study possible protective effects of BDNF on fetal rat ventral mesencephalic cells. Exposure to 100ng/ml BDNF for 7 days in vitro produced a dose-dependent recovery of dopaminergic neurons among the dopaminergic and cortical areas whereas the dopaminergic functions. Further experiments will determine whether BDNF has any effect on the development of the dopaminergic system.

IMMEDIATELY AFTER TERMINATION OF THE MPP+ TREATMENT, DA UPTAKE IN BDNF-TREATED CULTURES WAS REDUCED TO THE SAME LEVEL AS IN MPP+ -TREATED CULTURES. In order to isolate the chicken CNTF, we have synthesized both PCR primers and long oligonucleotides suggested by the available sequence data from mammalian CNTFs. A cDNA library prepared from the E18 chicken choroid, a rich source for the CNTF, was screened. Positive clones are now being sequenced in order to verify their identity.

REGULATION OF CNTF mRNA LEVELS IN RAT BRAIN CULTURES. P. Carroll*. M. Landreth, A.J. Gage, and D. Holtz. Dept. of Biology and Neurobiology, University of California, Irvine, CA 92717.

MOLECULAR CLONING OF NERVE GROWTH FACTORS FROM THE CHICKEN EMBRYO. A. Backstrom*, T. Hallbock, A. Kyllberg*. C. Bank, T. Larhammar, J. Renge, and H. Hedin. Dept. of Developmental Biology, and Department of Medical Genetics, (§). Uppsala University, Biomedical Center, S-751 23 Uppsala, and Department of Medical Biochemistry and Molecular Neurobiology (*), Karolinska Institute, Stockholm, Sweden.

The chicken embryo offers an accessible system for examining early developmental factors. Herein, we report the cloning of the NGF gene. NGF mRNA levels were determined in several developmental stages by Northern blot analysis and in situ hybridization. The results indicate that NGF mRNA levels are maximal at E14 and decrease rapidly thereafter. This finding suggests that BDNF accelerates the recovery of dopaminergic neurons from MPP+ toxicity.

REGULATION OF CNTF mRNA LEVELS IN RAT BRAIN CULTURES. P. Carroll*. M. Landreth, A.J. Gage, and D. Holtz. Dept. of Biology and Neurobiology, University of California, Irvine, CA 92717.

MOLECULAR CLONING OF NERVE GROWTH FACTORS FROM THE CHICKEN EMBRYO. A. Backstrom*, T. Hallbock, A. Kyllberg*. C. Bank, T. Larhammar, J. Renge, and H. Hedin. Dept. of Developmental Biology, and Department of Medical Genetics, (§). Uppsala University, Biomedical Center, S-751 23 Uppsala, and Department of Medical Biochemistry and Molecular Neurobiology (*), Karolinska Institute, Stockholm, Sweden.

Neurotrophin-3 (NT-3) is a structural homolog of NGF, which displays a restricted pattern of mRNA expression in adult rat brain, confined mainly to hippocampus. To reveal possible targets of NT-3 trophic activity within mammalian brain, receptor binding autoradiography was performed with purified, recombinant human 125I NT-3 labelled by the lactoperoxidase method. Approximately 2 moles of 125I was incorporated per mole NT-3 homodimer, and 125I NT-3 retained full biological activity as measured by chick embryonic node ganglion and sympathetic ganglion neuron survival assays. The pattern of specific binding of 125I NT-3 was compared with that of 125I NGF and displayed a broader distribution within one month old rat brain. Specific binding of 30 pM 125I NT-3 was observed in cerebellum and telencephalic sites, particularly hippocampus. Binding was blocked by increasing concentrations of unlabelled NT-3 but not by equivalent concentrations of NGF. These results suggest possible targets of NT-3 trophic activity within mammalian brain.


The mRNA's for the three known neurotrophins (NGF, BDNF and NT-3) are differentially distributed in mammalian CNS. Therefore, it is expected that at least some neuronal populations responsive to BDNF and NT-3 will be found to be distinct from those which respond to NGF. The prototypic neurotrophin, NGF, is a survival factor for the cholinergic neurons of the basal forebrain and it is retrogradely transported by these cells. This suggests that studies of axonal transport of NGF, BDNF and NT-3 might prove useful in identifying neuronal populations potentially responsive to these related trophic molecules. [125I]-BDNF, -NGF and -NT-3 (0.06-1.5 μCi in 0.02-0.5 μl) were injected into the hippocampus or striatum of adult, male rats. Sections processed for autoradiography showed robust transport of NGF from the hippocampus to the medial septum and the diagonal band; transport of BDNF and NT-3 to these cholinergic nuclei was also evident, though less marked. All 3 neurotrophins were transported to the ventral mesencephalon from the striatum. However, the distribution for BDNF and NT-3 was distinct from that seen for NGF. Furthermore, BDNF and, to a lesser extent, NT-3 were transported within several additional telencephalic and diencephalic cell groups which did not transport NGF. Neuronal populations which selectively transport BDNF and/or NT-3 may respond specifically to these members of the neurotrophin family under normal or pathological conditions.


Mechanical injury to the adult rat brain results in the accumulation of neurotrophic activity for cultured parasympathetic, sympathetic and sensory neurons at the lesion site (Nato-Sandkuhl et al., 1983. J. Neuroscience, 3: 2219-2228). In order to determine which neurotrophic factors are responsible for this activity, we have measured changes in the levels of mRNA for some neurotrophic factors in the hippocampus and cortex. We found that in the normal adult rat hippocampus and cortex, CNTF mRNA levels are relatively low but this increases dramatically after lesion by as much as 7 fold compared to sham operated controls. Areas distant from the lesion site show no increase in CNTF mRNA unless they are directly interconnected to the ablated areas, such as the regions of the neocortical cortex and hippocampus where mRNA levels are as high as at the wound site. In contrast, mRNA levels for the neurotrophins BDNF and NT3 were relatively high in normal adult hippocampus but fell by 2-3 fold after lesion both contralaterally and adjacent to the wound.


Rat ciliary neurotrophic factor (CNTF) has recently been molecularly cloned, purified to homogeneity and characterized (Panagotatos et al., 1991). Binding studies with [125I] CNTF on monoculture layers of purified rat hippocampal astrocytes demonstrate equilibrium binding to be reached following a 50 min incubation period at room temperature and to be stable up to at least 120 min. Scatchard analysis suggests that the [125I] CNTF binding is conformed to two sites. Experiments to biochemically characterize the ligand binding component of the CNTF receptor demonstrate a loss of specific [125I] CNTF binding following exposure (60 min at 37°C) to purified preparations of phospholipase C suggesting that a glycosyl-phosphatidylinositol anchor is involved in the attachment of the CNTF binding component to the plasma membrane. In addition to investigating potential second messenger mechanisms which may be activated as a consequence of the interaction of CNTF with its receptor, we have observed the induction of the mesenger RNA for the immediate early gene c-fos. Time courses of a maximum 2 fold increase in the c-fos mRNA occurs approximately 1 hour following a challenge with 50 ng/ml CNTF.
446.17 REDUCTION OF CNTF LEVELS IN SCHWANN CELLS IN DEGENERATING SCATIC NERVE. B. Friedman, N.Y., L.M. Helgren, J. Budge, T. Day, R. Yamada, P. Fink. Department of Cell Biology and Anatomy, James H. Coulombe, J.R. Nishi, and F. Eckenstein, Oregon Health Sciences University. Portland OR 97201. The adult rat sciatic nerve is a rich source of CNTF (ciliary neurotrophic factor) and a prototypical immunocytotoxicity staining indicates that most of the CNTF is present in Schwann cells. To determine if the integrity of Schwann cell axon contact is a prerequisite for high levels of CNTF expression, we have examined CNTF levels in degenerating and regenerating peripheral nerve. In adult rats, the sciatic nerve was transected and then examined histologically at a series of survival times from 1 day to 2 weeks. A second series of rats were prepared where the sciatic was crushed and examined after 2 week survival times. CNTF levels were assessed immunocytochemically with a polyclonal antibody generated against recombinant rat CNTF. CNTF mRNA was identified with in situ hybridization. Schwann cells in the proximal stump of a transected sciatic nerve remain densely stained by anti-CNTF antibody and continue to express CNTF message for at least 3 months. However, when axons are allowed to regenerate distal to a nerve crush then Schwann cells re-express CNTF protein. These results suggest that Schwann cell expression of CNTF is maintained by signals(s) from related axons and/or by the matutorial state of the Schwann cell.

446.19 EXPRESSION OF NEUROTROPHIC FACTORS IN THE AXOTOMIZED Facial NERVE SYSTEM. W. Testinffaft and K.C. Harrington*. Neuroscience Research Group, University of Calgary, Alberta, Canada, T2N 1N4. Following axotomy CNS neurons undergo significant cellular atrophy and cell death, whereas PNS neurons usually survive disconnection from their targets and do not show atrophy until later stages post lesion. It is generally believed that this is due to differences in the availability of trophic factors. For motoneurons trophic support may come from the peripheral nerve and/or from the neuronal microenvironment with its reactive glial cells. We have tested these possibilities by using Reverse Transcription followed by Polymerase Chain Reaction amplification to study the expression of bFGF and several other trophic factors in i) the normal facial nerve ii) the distal nerve stump after transection iii) the normal and iv) axotomized facial nucleus. Our preliminary results indicate increased expression of CNTF mRNA in the axotomized facial nucleus while the expression is reduced in the distal nerve stump (compared to normal nerve). bFGF mRNA was increased in the axotomized facial nucleus, but not detected in the peripheral nerve (both normal and distal stump).

These data suggest that the neuronal microenvironment may provide trophic support to the axotomized facial motoneurones. In situ hybridization analysis is under way to determine the cellular source within the facial nucleus. Supported by MRC and NCE "Neuronal Regeneration" (Canada).

446.20 A NEUROBLASTOMA CELL LINE REGULATES VIP GENE EXPRESSION IN RESPONSE TO CNTF. Aviva J. Symes, Shane E. Hyman and J. Stephen Eiken. Molecular Neurobiology Laboratory. Massachusetts General Hospital. Harvard Medical School. Charlestown. MA 02129. Ciliary neurotrophic factor (CNTF) was originally identified as a survival factor for embryonic neurons of the chick ciliary ganglion. CNTF has also been shown to promote the differentiation of cultured sympathetic neurons towards the cholinergic phenotype, inducing choline acetyltransferase activity and peptides containing the serine tyrosine-phenylalanine-lysine (STK) sequence. To determine whether CNTF would regulate expression of VIP in a neuronal cell line, CNTF treatment of a neuroblastoma-human neurotrophin-3 (NBFL) cell line was done. VIP mRNA levels were assessed immunocytochemically with a polyclonal antibody against recombinant rat CNTF. CNTF induction of VIP mRNA was detectable after 1 day of treatment and was sustained for at least 7 days. VIP mRNA levels were much lower in the absence of CNTF. CNTF treatment had no effect on VIP mRNA in NBFL cells but may enhance the effect of CNTF. Using VIP promoter-luciferase fusion genes we demonstrated that the effect of CNTF on VIP mRNA in NBFL cells is at least partially transcriptional. Two of 5 flanking regions of the human VIP gene were sufficient to confer CNTF responsiveness to these fusion genes. Increasing luciferase activity 20-fold in CNTF-treated, transfected NBFL cells. However, deletion which yielded a still active promoter was unresponsive to CNTF. The CNTF-responsive NBFL cell line will enable further investigation of the molecular mechanisms by which CNTF, through alterations in gene expression, promotes neuronal differentiation.

446.21 CLONING AND EXPRESSION OF GROWTH-PROMOTING ACTIVITY (GPA). A CILIARY NEUROTROPHIC FACTOR ISOLATED FROM ADULT CHICK SCATIC NERVES AND EMBRYONIC CHICK EYES. Parent, A.S., Cachagas. G., Lee A., Leong D.W., Nikolici, K. Eckenstein, F.P., and Nishi, K. Dept. of Cell Bld. & Ass. Oregon Health Sciences University. Portland OR 97210 and Dept. of Mol. Biol., Genex Inc. #50 Point San Bruno, So. San Francisco, CA 94080. Neurons in the chick ciliary ganglion (CG) undergo a defined period of cell death, during which sensory neurons in the ventral root are spared. The mechanism by which these two populations of neurons die is not known. We have prepared a cDNA library derived from embryonic day 15 chick eyes. A 1453 bp cDNA clone isolated which shows approximately 45% homology with mammalian sciatic nerve ciliary neurotrophic factor (CNTF). This relatively low degree of homology for a potential neurotrophic factor (EDOU-20 pg/ml) suggests that GPA may be a homologue of CNTF, and not merely the chicken version of CNTF. GPA, like CNTF, promotes neuronal survival in the chick ciliary ganglion. GPA is expressed in the choroid layer of the eye, which is one of the targets of CG neurons in vivo (Walker, et al., this meeting). In order to test whether GPA is a neurotrophic factor in vivo for CG neurons, and to determine the relationship between GPA and CNTF, we have examined the expression of GPA and CNTF genes separately and together. GPA gene expression was detected by northern analysis and in situ hybridization. We are also expressing a recombinant GPA to examine the in vitro and in vivo activity of the protein. Supported by NS31576 (R01) and AG07442 (P30).

Reportedly prevents lesion-induced degeneration of neurons within the facial nucleus of the neonatal rat. We have recently described the production and purification of recombinant human CNTF (Negro et al., 1991). A structural analysis of the CNTF molecule is critical to an understanding of its mode of action. Here we have utilized the B131 system and polymerase chain reaction to generate human CNTFs. Mutant CNTF DNA constructs were expressed in E. coli. The resultant CNTF proteins were isolated by SDS-PAGE, eluted and assayed for trophic activity on CNTF responsive ganglionic neurons. Several mutations directed at the hydrophobic core region of the protein yielded marked losses in biological activity. These findings are consistent with the observed alpha-helical nature of CNTF, and indicate that retention of this conformation is necessary for activity. Purification of these mutant human CNTFs now in progress will provide the material needed to accurately determine the 3-D structure of this trophic factor.


Embryonic spinal cord have provided useful results but are often costly. We report new results obtained by the combination of two other strategies: (1) purification of CNTF from chicken brain extract; (2) re-seeded cultures of purified motoneurons from P1 chick embryos in serum-free conditions, chicken MN survival is considerably enhanced by muscle extract, but not by other factors tested, including NGF, bFGF, IGF-1, and insulin. Levels of mRNA for such neurotrophic factors may therefore be regulated in muscle or nerve by the motoneuron itself.

This work was supported by A.F.M. and I.R.M.E.


Removal of spinal and supraspinal descending input to the lumbar spinal cord by a thoracic spinal transection performed on embryonic day E12 (deafferentation) results in a significant loss of lumbar motoneurons (MN) between E10 and E12. A small amount of naturally occurring MN loss (1500-2000 cells) also occurs between E10 and E12. To examine whether these losses reflect the deprivation of CNS-derived trophic agents, brain extract (BEX), astrocyte conditioned medium (ACM), and several purified proteins (bFGF, CNTF & IGF-I) were administered daily to neonatal rats (between E10 and E12). MN numbers were assayed histologically on E18. Crude and partially purified BEX, ACM and S100 prevented both the naturally occurring (E10-12), as well as the additional deafferentation-induced MN death, but were without effect on naturally occurring MN death between E10-E12. Surprisingly, partially purified muscle extract also prevented both types of cell death between E10 and E16. Although these data demonstrate that deafferentation-induced MN death can be prevented by CNS-derived proteins, the specific agents involved, their mode of regulation by afferents and their relation to muscle-derived trophic factors remain to be clarified.

447.1 Neuronal Death II

A THEORETICAL MODEL OF NATURALLY OCCURRING NEURONAL DEATH IN DEVELOPMENT. Lucia Galli-Resta and Giovanni Resta*, Istituto di Neurofisiologia, CNR 56127 Pisa, Italy and Istituto di Elaborazione dell’Informazione, CNR 56100 Pisa, Italy.

Throughout the animal kingdom, the formation of the nervous system involves the elimination of many cells. This naturally occurring cell death is observed in the majority of nervous structures and causes the loss of 30-75% of the neurons initially generated.

Elimination of erroneous projections, as well as proper size matching between connecting structures can be achieved through cell death. However many more cells die than it would seem necessary to eliminate erroneous projections and conflicting data are present for the survival and/or differentiation of the target and input structures. It is believed therefore that no unitary model can be presented to account for the phenomenon of neuronal death in different cases.

We have developed a simple theoretical model which accounts for naturally occurring neuronal death in development. The model quantitatively predicts the outcome of many of the experiments in the literature and is able to account for failures of the theory in a number of single framework experiments until now viewed as contrasting.

447.2 Intense Sound Releases an Amine Present in Perilymph and CSF. R.P. Bobbin and M. Fallon*. Kresge Hearing Research Laboratory, LSU Medical Center, New Orleans, LA 70112.

Unk 2.5 is an amine released by 50 mM K⁺ into effluent from the perilymph compartment of the cochlea (Bobbin et al., Hear. Res. 46: 83, 1990). We examined the effect of sound on levels of Unk 2.5 in chick cochlear effluent. Using anesthetized guinea pigs, the perilymph compartment of the cochlea was perfused with an artificial perilymph containing low Na⁺. Effluent was collected before, during and after exposure of the ear to intense sound. Amines in the effluent were measured utilizing precolumn derivatization with o-phthalaldehyde/2-mercaptoethanol and HPLC. Broad band sound (10 min at 118, 124 and 130 dB SPL) increased the levels of Unk 2.5 without affecting the levels of glutamate and 15 other amines in the effluent. We call Unk 2.5 "nois induced release amine," or "niramine". Niramine eluted almost at the same time as d-3,4-dihydroxyphenylglycine, phosphorosine, cysteic acid, cysteine sulfinate, homocysteine sulfinate, and homocysteic acid. In CSF, niramine was found in higher concentrations than in plasma (5:1) and at about the same relative fluorometric values for the two. The role of niramine is unknown. (Supported by NIH grant DC-00379).

A common feature of apoptosis is the cleavage of cellular DNA into fragments with sizes that are integral multiples of 100 bp (nucleosome repeat). DNA fragmentation is normally the initial event that occurs because apoptosis is inhibited by aurintricarboxylic acid (AT) — a drug that, among its many other effects, is a well-known inhibitor of DNA fragmentation during cell death in rat sympathetic neurons, in neuronal PC12 (nPC12) cells, and in chromaffin-like PC12 (cPC12) cells by observation of purified cellular DNA on agarose gels stained by ethidium bromide or, if greater sensitivity was required, by transfer to nylon filters which were probed with [32P] labeled rat genomic DNA. DNA fragmentation was inhibited by transfer of 100 ng of PC12 cells to serum-free, NGF-free medium or transfer of sympathetic neurons to NGF-free medium. No evidence of DNA fragmentation could be observed in these cells at any time up to 72 h after initiation of cell death (by 72 h most of the cells are dead). Electrophoresis of the DNA on denaturing gels also showed no detectable increase in single-stranded breaks. Similar assays performed using glucocorticoid-treated rat thymocytes showed typical DNA fragmentation, indicating that our assay system is capable of detecting neuronal DNA fragmentation were it to be present. Nevertheless, AT strongly inhibited cPC12 cell death and had a similar, albeit weaker, effect in nPC12 cells and neurons. Another difference between nPC12 and cPC12 cells is that cell death in the former, but not in the latter, was partially inhibited by protein synthesis inhibitors. We conclude that DNA fragmentation, whatever its role in neuronal cell death, is not required for neuronal apoptosis. Furthermore, inhibition of apoptosis by AT, a very general inhibitor of protein-nucleic acid interactions, can't be taken to imply that nuclease activity is necessary for apoptosis. Finally, we suggest that nPC12 and cPC12 cells do not proceed by identical mechanisms, with a transcription-dependent process playing a role in nPC12 cell death as one does in neuronal death.

447.5 DECREASE IN THALAMIC AND NEOCORTICAL VOLUMES AFTER A SMALL PRENATAL CORTEX LESION IN THE CAT. L.D. Loopnljt, L.R. Villanueva, R. Gresch, and D.A. Henry. Center and Divisions Lab Animal Medicine, UCLA, Los Angeles, CA 90024.

In monkeys, prenatal fetal cortex lesions have little effect on behavior or neuronal cell packing density (CPD) in thalamic medio-dorsal nucleus [Brain Res. 152(1978)451]. In cats, lesion-induced degeneration is less in neonatal-external ventrobasal (VBX) and basal ventromedial (VMB) nuclei neuronal morphology and position within the motor pool. Nevertheless, AT strongly inhibited cPC12 cell death and had a similar, albeit weaker, effect in nPC12 cells and neurons. Another difference between nPC12 and cPC12 cells is that cell death in the former, but not in the latter, was partially inhibited by protein synthesis inhibitors. We conclude that DNA fragmentation, whatever its role in neuronal cell death, is not required for neuronal apoptosis. Furthermore, inhibition of apoptosis by AT, a very general inhibitor of protein-nucleic acid interactions, can't be taken to imply that nuclease activity is necessary for apoptosis. Finally, we suggest that nPC12 and cPC12 cells do not proceed by identical mechanisms, with a transcription-dependent process playing a role in nPC12 cell death as one does in neuronal death.

447.7 DEVELOPMENTAL CHANGES IN THE ARRANGEMENT OF MOTOR POOLS IN THE CHICK SPINAL CORD MAPPED USING FLUORESCENT BEADS. R.V. Birling and D. Summerbell. Department of Biology, Open University, Milton Keynes MK7 6AA, UK and National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

Each muscle of the vertebrate limb is innervated by a cluster of motor neurons in the spinal cord, but there is little similarity between adjacent pools. These are usually mapped using retrograde filling techniques in which single muscles are injected with tracers, but such methods do not reveal the overlap between adjacent pools. In the present experiments fluorescent latex microspheres (Lumaflex inc) were used to retrogradely fill motor neurons. The position of the cord was plotted in three-dimensions using a computer-aidered drawing system (Interactions Company). The advantages of using these beads are that they are very easy to count, they are not fluorescent and all colours are transported in an identical manner. The advantages of the computer-aidered system is that the position of the filled cells can be mapped in the intact cord, thus verifying problems relating to sectioning initially the pools are diffuse and overlapping but gradually become more discrete. We suggest that selective cell death is responsible for this change. This could not have been detected using single injection tracers. The method of filling and the opportunity of injecting motor neurons with dyes to study the relationship between morphology and position within the motor pool.

447.4 ABLATION OF THE THORACOLUMBAR NEURAL TUBE IN CHICK EMBRYOS PROMOTES SURVIVAL OF CEREBRAL PREFrontAL NEURONS. P. Cawthorne, O.C.K., Toronto, Ont., Canada.

In chick embryos two distinct columns of prefrontal neurons develop from a common primordium and establish structural contact with sympathetic trunca ganglia (STG): (1) a thoracolumbar column of SN (CI,) normally forms permanent synaptic contacts with the prefrontal cortical column (CC) develops initial contact with STG early in development but disappears by postnatal day 4.5. The inability of CC neurons to form permanent contacts with STG neurons during normal development appears to be a characteristic intrinsic to CC cells alone, since neurons of the thoracolumbar CT transplanted into the cerebral neural tube region do survive contact with the STG and maintain contact to at least day 16 (Soc. Neurosci. Absts. 14(2):897).

To test the possibility that CC neurons have the potential to establish stable structural contacts with STG the thoracolumbar neural tube was removed surgically at day 2.5E prior to neurite outgrowth. In these experimental (TL-Rem) embryos the cervical neural tube was left intact, while postganglionic SN neurons developed in the absence of their normal innervation. Analysis of urea silver nitrate stained serial cross-sections of day 8E experimental (TL-Rem) embryos revealed that after neural tube removal STG develop normally and primitive CC neurons now survive and maintain contact with STG. Thus, CC neurons have the potential to form stable contacts with STG neurons. Current analysis will determine if these connections are maintained beyond day 8E and define the pattern of neurite outgrowth into STG in experimental (TL-Rem) embryos.


We have been examining the expression of ubiquitin, a protein associated with cell death in insects, during the death of motoneurons and neural crest derived neuromening of the chick embryo. Northern analysis has demonstrated that polyubiquitin transcripts increase in the spinal cord coincident with neuronal death. In situ hybridization is being used to determine the source of the mRNA. Western blotting experiments showed that ubiquitin, isolated from the cervical cord contained a greater percentage of total spinal cord protein at early vs. later stages of the embryo. Immunohistochemistry has revealed possible site of expression: increased ubiquitin immunoreactivity (IR) was present over the entire lumbar lateral zone of the neural tube. These data suggest that polyubiquitin may play a role in developmental cell death in the vertebrate nervous system.
MOTONEURON CELL DEATH IN THE NUCLEUS AMBIGUUS AND EPIGLOTTIC NUCLEUS OF RATS: CRITICAL PERIODS IN THE DEVELOPMENT OF MOTONEURON DEATH

THURSDAY AM NEURONAL DEATH II 1125

447.10 NEURONAL BIRTH AND LOSS IN DEVELOPING RABBIT RETINAE

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We have investigated the spatio-temporal patterns of cell birth and loss in the retinae of developing albino rabbits. Under Halothane and nitrous oxide anaesthesia, 4th-7th retinæ were injected in foetuses on either the 13th post-conceptional day (13PCD), 18PCD or 29PCD. The mother and babies were sacrificed with an overdose of barbiturate between 20PCD and 34PCD. The young retinæ were embedded in paraffin, sectioned into 4μm sections, and stained with hematoxylin and eosin. The number of labelled cells were made of large, medium and small subtypes, either in the developing retinae as a whole, or in specific regions.
448.5 THE DEVELOPMENT OF VIP mRNA EXPRESSION AND VIP RECEPTOR BINDING IN THE RAT CNS. J.M. Hill, A.C. Tamner*, D.V. Agapian and D.B. McGinn. Laboratory of Development and Neurobiology, NICHD, NIH, Bethesda, MD 20892.

VIP has neurotrophic properties (PNAS 83:1159,1986) and a role in neurobehavioral development (Peptides 12:187,1991). The present work traces the development of VIP with in situ hybridization and receptor binding to both GTP-sensitive and insensitive sites with in vitro autoradiography in rat embryos from E14 to P14 and adult CNS.

The mRNA for VIP was expressed at very low levels until P8, however, by E14 it was seen in the cortex. By P8, message increased and was additionally found in the thalamus, superiorcicums nucleus (SCN), substantia, superior colliculus and dorsal raphe. By P14, message was greatest in the cortex than the thalamus, but in the adult, the SCN and thalamus had the greatest levels.

VIP binding was uniform and dense in the brain stem and spinal cord by E14, but localized to specific cortical and hippocampal layers. By E16, VIP binding was also seen in the SCN, thalamus, pinael and dorsal raphe. By E19, VIP binding was seen in the diagonal band, septum, cerebellar peduncles and hypothalamus. In D8-D14 VIP remained uniform and dense in the brain stem and basal forebrain and midbrain structures but in the cortex and hippocampus was limited to specific layers. In the adult, VIP binding was localized to specific cytoarchitectural sites throughout the brain. As in the adult, most VIP binding sites were sensitive to displacement by GTP, however, as early as E19 GTP-insensitive binding sites were observed in the spinal cord and thalamus.

VIP receptor binding develops earlier and is more widespread than VIP mRNA. Although changes in relative abundance occur in both throughout development, by P8 VIP and its receptors are colocalized in several brain regions and remain in the adult.

448.6 REGULATION OF IGF-II GENE EXPRESSION DURING NEUROBLASTOMA PROLIFERATION AND DIFFERENTIATION. D.M. Martin, R.O. Ouster* and E.L. Fielding, Department of Neurology and Mental Health Research Institute*, University of Michigan Medical School, Ann Arbor, MI 48109.

Insulin-like growth factor-II (IGF-II) belongs to the insulin family of polypeptides and has been implicated in neuronal mitogenesis. Both primary neuroblastomas and some cell lines have been shown to produce large amounts of IGF-II, which can act as an autocrine growth factor (El-Badry, O. et al., J Clin Invest 84:829-839, 1989). Similarly, reduction of IGF-II mRNA levels during neuronal proliferation and differentiation. In this study, we report decreased IGF-II gene expression in differentiated neuroblastoma cells.

We have found by Northern analysis that the clonal neuroblastoma line SH-SY5Y expresses high amounts of IGF-II mRNA. Neuroblastoma cells differentiate in response to interferon-gamma (IFN-g) (Watanabe, H. et al., Jpn J Cancer Res 80:1072-1076, 1989). We observed growth arrest and gross morphological changes in SH-SY5Y following addition of IFN-g. We analyzed IGF-II mRNA levels in SH-SY5Y cells maintained in the presence or absence of IFN-g. Under these conditions, IGF-II mRNA levels decreased over a period of 3 or 6 days. These data suggest that IGF-II gene expression is correlated with neuronal differentiation.

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448.7 NMDA ACCELERATES AND AMPLIFIES THE ONTOGENY OF SOMATOSTATIN IN MONOLAYER CULTURES OF FETAL RAT TELENCEPHALON. R.J. Robbins, and S. Welsh, Neuroendocrinology Program, Departments of Medicine and OBGYN, Yale Univ. School of Medicine, New Haven, CT 06510.

The survival and differentiation of mammalian neurons depends in part on the stimulation which they receive during a critical developmental period. We have previously reported that telencephalic SS interneurons in vitro display enhanced susceptibility to excitatory amino acids (EAA) only after they become mature. For the first 11 days in vitro cortical SS neurons obtained from 16 day old embryos are not killed by NMDA. We hypothesized that EAA would still bind to immature SS neurons and alter their in vitro response. Fetal rat telencephalic cultures were grown for 20 days in the presence of various concentrations of EAAs. The SS content of the cells was measured byRIA. NMDA (100 μM) added at the time of plating increased cellular SS content by 130% at day 5, by 180% at day 10, and by 50% on day 20. Equimolar doses of Quisqualic acid resulted in a significantly greater than 80% decrease in SS at all time points. AMPA (10-100 μM) significantly increased SS content at days 10,15, and 20. The NMDA blocker APV, retards early SS development and results in increased SS content by days 20 and 27. In summary, EAAs can exert dramatic effects on the development of somatostatinergic neurons in vitro. We conclude: (1) that the normal development of SS interneurons in vivo may depend in part on their exposure to glutamate and, (2) that functional EAA receptors exist on neurons prior to their full maturation.

449.1 NEUROMAGNETIC ASSESSMENT OF SOMATOSENSORY CORTICAL ORGANIZATION FOLLOWING PERIPHERAL NERVE INJURY AND RECONSTRUCTION. J. Manning, L.U. Uhlrich, R.W. Lado, J.L. Girmannt and R.R. Linds Department of Physiology and Biophysics, NYU School of Medicine, NY, NY 10016.

A 14-channel neuromagnetic recording system is being used to evaluate changes in cortical organization following peripheral nerve injury and surgical repair in a number of subjects. Studies in primates (Wall et al, J Neurosci 1986 8(21):233) have shown that following peripheral nerve transection and repair, the somatosensory cortical maps reorganize over a period of time during the repair process. The high spatial resolution of the MEG, on the order of a few millimeters, supports the idea that cortical plasticity following peripheral nerve injury and surgical repair in a number of patients is due to the plasticity of the nerve-ending and also to the plasticity of the developing spinal cord and thalamus.

449.2 A PERIPHERY-RELATED PATTERN IS EVIDENT IN RAT SOMATOSENSORY CORTEX AT BIRTH. B.L. Schoppa and D.O. O'Leary, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla CA 92037.

The "barrels" of rat somatosensory cortex (S) are arranged in a pattern isomporic to the distribution of vibrissae on the body surface. A host of techniques have shown that barrels emerge postnatally and are distinct by the end of the first postnatal week. The timing and sequence of events that lead to the differentiation of barrels are crucial to defining the agents that instruct their formation. Ventrobasal thalamocortical (VB) afferents are hypothesized to control barrel differentiation and the somatotopic patterning of the neocortex. To understand the agents that define the emergence of barrel patterning of VB afferents in S in rats at age (P4) (P0 is the first 24 hrs after birth). We assessed for ACHE, an early marker of VB afferents (Kos JCN '79; Neuron '89), and directly labeled VB afferents with Di in fixed brains. ACHE histochemistry, a more sensitive method, is used in animals at age (P16 to P4). (P0 is the day of inamination. Di is the first 24 hrs after birth). We assure for ACHE, an early marker of VB afferents (Kos JCN '79; Neuron '89), and directly labeled VB afferents with Di in fixed brains. ACHE histochemistry, a more sensitive method, is used in animals at age (P16 to P4). (P0 is the day of inamination. Di is the first 24 hrs after birth). We assure for ACHE, an early marker of VB afferents (Kos JCN '79; Neuron '89), and directly labeled VB afferents with Di in fixed brains. ACHE histochemistry, a more sensitive method, is used in animals at age (P16 to P4). (P0 is the day of inamination. Di is the first 24 hrs after birth).
449.3 DEVELOPMENT OF THALAMOCORTICAL TERMINAL ARBORS IN LAYER IV OF MOUSE BARREL CORTEX. A. Agram and L.M. Jones. Dept. of Anatomy and Neurobiology, College of Medicine, Irvine, CA 92617.

Thalamocortical axon terminals in the adult mouse somatosensory cortex are highly ordered. Radially they are segregated into a diffuse tier in the deep layers and a dense tier in the superficial layers. The upper tier is parcellated into discrete patches, each at the core of a cluster of cells or “barrel”. In the newborn mouse layers IV and V are still undifferentiated and thalamocortical terminals are restricted to layers VI/IV. The formation (around postnatal days 3-4) of the barrels with their associated thalamocortical arbors is a complex morphogenetic event that is not yet fully understood. Of particular interest is whether the axonal clusters in layer IV arise from an initial diffuse projection by elimination of surplus branches or whether they are formed de novo as discrete entities. We studied this question by placing several particles of Di-I in the thalamus and fixed 400μm thick thalamocortical slices (Agram and Connors, Neurosci. Lett. 165, 375-379, 1991) prepared from animals at different ages during the first two postnatal weeks. The dye was allowed to diffuse for 1-4 weeks at 37°C and the slices imaged as whole mounts using a laser confocal microscope. At postnatal day 2 (P2) most all labeled branches were in layers VI and V, with the branches coursing up through the cortical plate (presumptive layers II-IV). By P4 individual axons were seen to course radially upwards through upper layer V without branching and upon entering lower layer IV abruptly fanned out into several branches; a few of several axons overlapped to form a cluster, and adjacent clusters were clearly separated. The number of layer IV branches per axon increased dramatically over the next 10 days but the clusters remained well-defined. Our data are consistent with the hypothesis that the terminal arbors in layer IV are segregated from their very beginning and that new branches are added within their “correct” cluster.

449.5 AGE-RELATED DIFFERENCES IN SI CORTEX RESPONSE LATENCIES AFTER SOMATOSTIMULATION OF THE FACE, FOREPAW, AND HINDPaw IN THE NEONAT AL RAT. C.A. McCandlish, C.X. Li*, and R.S. Waters. Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163.

Using natural somatic and electrical stimulation, we reported that evoked responses could be elicited from SI cortex of newborn rats prior to PND-7 and that the functional development of the barrel map followed normal developmental gradients. We extend these findings to include a description of the latencies of evoked responses over the first 14 postnatal days. Neonatal rats, ranging in age from PND-1 to PND-14, were anesthetized with Nembutal (40mg/kg ip) and placed on a heating pad. A midfacial incision was made overlying the skull, the skin retracted, and the cisterna magna opened. The area of skin overlying SI was removed and the dura opened. Carbon-fiber electrodes (1-2 MΩ) were used to record mechanically and/or electrically evoked responses from SI after stimulation of regions of the contralateral face, forepaw, and hindpaw. At the end of the experiment, electrolysis lesions (3-4°/sec) were made over selected sites, and animals sacrificed. Hemispheres were removed, sectioned coronally (40μm), and stained with cresyl violet stain. Beginning on PND-1, response latencies were measured and compared between each SI cortical subfield area that showed latency periods are shortest after stimulation of the face, followed by forepaw, and then hindpaw region. Latencies were measured from each subfield’s region decreased over each successive postnatal day. (Supported by NSF Grant BNS 88-02766)


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Vibrissectomy induces plastic change in the adult rat barrel cortex associated with a persistent increase in GAP-43 mRNA and protein. The immunohistochemical label for GAP-43 appeared first in layer IV, 1-7d after total unilateral vibrissectomy with sparing of C3. Autoradiograms were generated from brain sections hybridized with labeled mRNA in situ. Autoradiographic strips were analyzed by computer to determine relative hybridization intensity. Measurements were taken from animals sacrificed. Hemispheres were removed, sectioned coronally (40μm), and stained with cresyl violet stain. Beginning on PND-1, response latencies were measured and compared between each SI cortical subfield area that showed latency periods are shortest after stimulation of the face, followed by forepaw, and then hindpaw region. Latencies were measured from each subfield’s region decreased over each successive postnatal day. (Supported by NSF Grant BNS 88-02766)

449.8 POST CRITICAL PERIOD PHENOMENA IN CORTICAL BARREL FIELDS IN ORGANOTYPIC SLICE CULTURES FROM RAT SOMATOSENSORY CORTEX. J.M. Behan, A. Koerker, and J. Bult, Friedrich-Miescher-Labor der Max-Planck-Gesellschaft, 7400 Tübingen, Germany. Present address: Department of Comparative Biosciences, University of Wisconsin, Madison, WI 53706, USA.

During early postnatal development, the functional architecture of barrel cortex is a critical period when alterations of the sensory periphery disturb barrel formation. However, peripheral manipulations after the critical period appear not to affect the barrel architecture. In this study, we report that the mechanisms behind these phenomena remain intact and are, yet unknown. Although there is evidence that thalamocortical afferents play a role in shaping barrel fields, inputs from other subcortical and cortical sources may be equally important. We have begun to study barrel fields in organotypic slice cultures, where all of these inputs can be eliminated. First, we determined whether barrels could be maintained in the absence ofafferent input, and secondly, in the absence of input from other cortical layers. Slice cultures of the somatosensory cortex were prepared from P3-P4 rat brains, by which time barrel fields were formed. Cytochrome oxidase histochemistry and Nissl staining were used to identify the presence of barrels in slice cultures. In sagittally cut slices, barrels were observed in cortical layer 4 up to 10 days in vitro (DIV), the longest period examined. In tangentially cut cortical slices, after 1 DIV, barrels could usually be identified. However, after 2-4 DIV cells in the barrels reorganized, and cells from the barrel wall moved into the barrel hollow. After another 1-2 DIV, the remaining barrel area disappeared and the cells were homogeneously distributed in the slice culture. Thus, intact connections within cortical columns appear to be necessary to sustain the functional architecture of barrel cortex in vitro. These results suggest that interactions between cortical layers may play a role in the termination of the critical period.
449.9

THE PRODUCTION OF CRYPTOPIA IN OPOSSUM PUPS FOLLOWING LESIONS OF THE TRIGEMINAL GANGLION. D.M. Feinberg* and B.L. Masters, Dept. of Nuclear Medicine & Radiology, Penn. State Univ., M.S. Hershey Med. Ctr., Hershey, PA 17033

During the course of studies on the impact of lesions of the trigeminal ganglion in opossum pups, we observed that the auricles on the side of the lesion were small and misshapen, i.e. evidence cryptopia. The auricle develops under a layer of mesenchymal cells separated from the skull by a plate of stratiﬁed epithelium that is directly continuous with the meatal plug. This auricular plate is present at birth. Progressive enlargement undergoes keratinization in the center dividing the posterior surface of the auricle from the skin overlying the cartilage of the auricle. The separation of the auricular plate typically begins at 12-15 days of age. We created lesions of the trigeminal ganglion using a microsurgery on pups attached to their mother at 1 day of age with halothane anesthesia. The condensing mesenchyme of the auricle and the auricular plate were consistently smaller and keratinization delayed on the side of the trigeminal lesions. By 21 days, grossly smaller auricles could be recognized in all experimental cases. We conclude that afferent nerves have a critical role in the differentiation of the auricular plate as other cutaneous appendages, and trigeminal lesions produce cryptopia that resembles unilaterality. Supported in part by USPHS Research Grant NS 19462.

449.10


PrV cells undergo a dramatic postnatal regressive process (Hobart et al., Neurosci. Abstr. 15: 99). Denervation, branching, and appandages are reduced to produce a simple, truncated, and polarized tree that is restricted to one "barrellet." We asked whether target destruction alters this process. Four newborn rats received electrocautery lesions of the right thalamus, wherein "destabilizing" PrV of 59 ± 27% (mean ± SD) of their cells (Jacquin et al., Neurosci. Abstr. 16: 90), yet sparing primary afferent projection patterns (Chiaia et al., JCN 203: 91). In adulthood, standard Golgi staining and computerized image analysis methods were used to compare dendritic trees of 50 experimental (E, contralateral to lesion) and 50 control (C, ipsilateral) rats. Importantly, these rats did not differ in the overall distribution of afferent input (E: 5 ± 2,s 9; C: 4 ± 1,10 μm²) and the # of primary dendrites (E: 1 ± 0.3, 7; C: 1 ± 0.3, 7). In adulthood, standard Golgi staining and computerized image analysis methods were used to compare dendritic trees of 50 experimental (E, contralateral to lesion) and 50 control (C, ipsilateral) rats. Importantly, these rats did not differ in the overall distribution of afferent input (E: 5 ± 2,s 9; C: 4 ± 1,10 μm²) and the # of primary dendrites (E: 1 ± 0.3, 7; C: 1 ± 0.3, 7). In adulthood, significant increases in total dendritic length (E: 588 ± 649, C: 774 ± 599 μm), # of dendritic branch points (E: 16 ± 12,9; C: 9 ± 7,6), and # of spines or swellings (E: 23 ± 1,3, S; C: 13 ± 1,7,6): Important measures that did not differ were transverse area of influence (E: 22,498, C: 20,805 μm²) and # of primary dendrites (E: 4,5, C: 4,4). These data suggest that near-normal patterning of afferent inputs is consistent with normal development of PrV cells. Other factors, such as interactions among dendrites and retrograde signals from the target, may also play important roles. DE07762, DE07734, DE08941.

449.11


We & De Permentier (in press) have shown that the central projections of non-infraorbital (IO) whiskers occupy supranormal areas in the rat brainstem after infraorbital injury at birth. We (Neurosci. Abstr., 16: 90) reported that such plasticity is not solely due to peripheral regeneration, error patterns, or projection remodeling of an immature projection pattern. Anatomical and physiological tools were used to study the time course and mechanisms underlying this phenomenon. Cytosine-oxide patches representing the 2 superopolaricular (SO) trigeminal subnuclei were reliably larger than normal postnatal day (P) 5 (1±1.5, 11 Control), but had not achieved adult-like disparity by P3 (1±1.5, 36 X Control). To determine whether patch enlargement is due to relative changes in impulse activity produced by nerve section, retetrodotoxin (N=3) or bupivicaine (N=4) were applied from birth to block infraorbital nerve impulses during the first 5-7 Ps. As previously reported, infraorbital nerve sections normalized the # of IO nerve axons proximal to the injury target, may also play important roles. DE07762, DE07734, DE08941.

449.12

DAILY WHISKER TRIMMING FROM BIRTH MIMICS NERVE INJURY-INDUCED RESPONSE ALTERATIONS IN TRIGEMINAL SECOND-ORDER CELLS. M.F. Jacquin*, Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

To assess experimental contributions to developing response profiles in subicularis interopolaris, 343 single units were studied in 10 rats after 6-9 weeks of left mystacial whisker trimming. Data were analyzed in 385 control cells. Encounter rates and whisker maps were normal. However, receptive field (RF) size, character and higher-order inputs were abnormal (neonatal infraorbital nerve cut data also shown, N=478; from JCN 126: 36). These data suggest that near-normal patterning of afferent inputs is consistent with normal dendritic development of PrV cells. Other factors, such as interactions among dendrites and retrograde signals from the target, may also play important roles. DE07762, DE07734, DE08941.

449.13


In order to identify the neurons of origin and to study the ontogeny of the lateral spinothalamic and trigeminonothalamic tracts (LSTT and TSTT, respectively), two retrograde labels, WGA-HRP (5%) or green fluorescent latex microspheres were used. Rat fetuses of 17, 18, 19 (FD-17, FD-18, FD-19) days of gestation were used in each case. The lateral posterior nucleus (VPL) or the ventrobasal complex of the thalamus unilaterally. All injections were made through the use of the suture landmarks of the skull and penetrated the urothel and articular membranes. After a 24 hour survival period, a caesarian section was performed and all animals were perfused intracardially. Last year at this meeting it was reported that spinal cord cells were labeled in the contralateral dorsal horn of postnatal day 1 day and 10 rats pups. In the FD-19 rat, both methods yielded labeled cells in the same pattern as postnatal day rats with the neurons of the lateral superficial lamina (lamina III) were absent and only neurons of the contralateral ventromedial dorsal horn were labeled. The (pliatal) medial lemniscus and most lateral principal sensory trigeminal nucleus were also labeled following VLP injections. Results in the younger animals so far demonstrate that both sensory systems, the trigeminonothalamic and TSTT are early developing tracts that are present prenatally in the rat.

449.14


A large # of trigeminal (V) ganglion and brainstem cells die after neonatal infraorbital (IO) injury, producing shrinkage and permanent deafferentation of the V brainstem complex. Yet, the cytoarchitecture of layers I and II of the medullary dorsal horn is selectively preserved. As a first step in characterizing the anatomic reorganization subserving such "plasticity" in the dor­sal horn, random systematic, and SEM stereologic methods were applied to layers I and II 90 days after cutting the left IO nerve at birth in 2 rats. To count synapses and terminals, as well as to compute a terminal arboration factor, the double distance correction factor, and percent counting efficiency, were used, respectively. In each case the # of IO axons proximal to the point of neonatal injury were also determined. Case 1 2 Case 1 2 Case 1 2 Case 1 2 Case 1 2

![Table:](https://example.com/table.png)

# of infrorbital axons (2 cases listed) 8,281,9,737 36,236,
# of synapses/100 μm³ 27,5, 28,2 22,2,5, 22,5,
# of terminals/100 μm² 36,0, 37,5 32,9, 28,3,
# of terminals with dense core vesicles/100 μm² 14,8, 15,5 13,1,5, 15,6,
# of terminals with round vesicles/100 μm² 18,1, 18,8 13,8, 10,8,
# of terminals with flat vesicles/100 μm² 3,6, 3,3 5,4, 1,5,
# of terminals with ring vesicles/100 μm² 14,9, 17,1 16,7, 14,9

These data suggest that the most normal #s of terminals and synapses exist in layers I and II, despite the fact that only 25% of IO axons survive axotom­y at birth. The selective preservation of inputs to layers I and II may reflect a higher % survival of ganglion cells that normally project here, maintenance of immature projections, or sprouting. DE07734, DE07662, NS23805.
449.15
NDF AND PERIPHERAL IMMUNOREACTIVITY DEFINE DISTINCT CLASSES OF RAT SENSORY GANGLION CELLS. M.E. Goldstein, S.B. Bashe and M.G. Gainer. Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.
We have used double immunofluorescence techniques with antibodies against NDF and peripherin to identify peripherin positive, NFL positive, and peripherin-NFL positive subpopulations in adult and neonatal (PN2) DRG in vivo. In addition, we have determined the proportion of the subpopulations that contain neuropeptides, substance P and calcitonin gene-related peptide (CGRP). Finally, we have determined which of these subpopulations are faithfully maintained in tissue culture. These studies have revealed three distinct subpopulations of neurons in rat dorsal root ganglia (DRG). In the adult rat, 46% of the DRG neurons were small and peripherin positive (NFL-IgG negative), and 48% were large and NFL-IgG positive (peripherin positive). About 6% were both peripherin and NFL-IgG positive. All of the DRG neurons marked with antibodies to NF-M and neuropeptide-Y showed bands of silver staining at positions corresponding to antibodies to NF-IgG and NFL-IgG. The neuropeptides were predominantly found in the small peripherin positive cell population. Eighty-seven percent of the peripherin positive small cell population contained substance P immunoreactivity, while 45% of the cell population contained CGRP. In contrast, only 18-24% of the NFL-IgG positive, large cell population contained neuropeptides and these were primarily in a smaller sized subpopulation. Similar patterns of antigen representation were observed in neonatal (PN2) DRG cell populations. Tissue cultures of sensory ganglion cells from PN2 DRG, in serum free medium, stably maintained exclusively peripherin positive neurons, with about five percent of these containing coexistent NFL-IgG immunoreactivity. Very high levels of neuropeptide gene expression were exhibited by these postnatal neurons in culture. In future experiments we hope to confirm these results using in situ hybridization and to examine the effect of growth factors on the phenotypes of DRG neurons in culture.

449.16

The age at which a fetus is capable of receiving and experiencing sensations delivered by noxious or tactile stimuli remains uncertain. In this study we have used electrophysiological and anatomical techniques to examine the development of some of the cutaneous receptors and their afferent fibres in fetal sheep (28 weeks of gestation) from 83 to 143 days of gestation (term = 146). At each age, recordings were made in the dorsal root ganglia of fetuses at various stages of development in order to assess the response properties of these afferents to brushing, touching and pinching the skin of the hindlimb. At the conclusion of the experiment, the fetuses were perfused and the skin from the skin, dorsal root ganglia and spinal cord stained for immunoreactivity for substance P (SP) and calcitonin-gene related peptide (CGRP). Samples of skin were also processed for silver staining to demonstrate nerve fibres. The earliest responses, recorded at day 87 resulted from activation of high threshold, rapidly adapting mechanoreceptor afferents. By 97 days, afferents responded to all of the above stimuli and had well defined peripheral receptive fields. Immunoreactivity for SP and CGRP was detected in the dorsal horn as early as 83 days. Wool follicle innervation and fine nerve endings in the skin were present in silver-stained sections at 87 days. With increasing gestational age, cutaneous innervation increased in density and complexity and the responses to stimulation were more readily elicited. These results suggest that cutaneous receptors are functional and afferent input is reaching the spinal cord as early as sixty percent of the way through gestation in the sheep.

DEVELOPMENT OF SENSORY SYSTEMS II

450.1
PARALLEL DEVELOPMENTAL CHANGES IN HIPPOCAMPAL PKC AND ELECTROPHYSIOLOGICAL PROPERTIES OF CA1 PYRAMIDAL CELLS. J.V. Sanchez-Palos, L. Chisholm, M. Joyce, and J. Rawson. Dept. of Psychology, Hunter College-CUNY, New York, NY 10021.
Visual system projections from the retina (RHT) and the lateral geniculate nucleus terminate in the ventrolateral portion of the suprachiasmatic nucleus (SCN) and entrain the circadian activity of SCN neurons. In the 3 day old rat pup visual system synapse maturation is not observed in the SCN, but by 4 days of age, RHT synaptic contacts are present. By 10 days of age, the number of SCN synapses is similar to that of the adult. In the present study we examined the appearance of Fos in the SCN of rats, 3 to 35 days of age. The avidin-biotin-HRP method was used with the primary antibody, rabbit-anti-Fos (Oncogene Sciences) diluted 1:10,000. In the ventrolateral SCN, developmental changes in activity, as indicated by the presence of Fos-LI, were observed that coincide with the synaptic input to the visual system. Three day old pups showed essentially no Fos-positive nuclei in the ventrolateral SCN. By 4 days of age, a few Fos-positive nuclei were observed and the number of labeled nuclei increased as the animal matured. At all ages, large numbers of Fos-positive nuclei were present in the dorsomedial SCN and this number also increased as the animal matured. Neither naturally occurring eye opening at 14 days of age nor surgical eye opening at eight days of age changed the activity of SCN neurons significantly. The results suggest that Fos appearance coincides with synaptogenesis in this system.

450.2
CALCIUM CURRENTS IN FETAL HIPPOCAMPAL NEURONS IN PRIMARY CULTURE FROM THE TRISOMY 16 MOUSE, A MODEL FOR DOWN SYNDROME. Z. Galdzicki, E.J. Coan, & S.I. Rapoport, LNS, NIA, NIH, Bethesda, MD 20892.
The entry of calcium through voltage-gated membrane channels plays a crucial role in a variety of hippocampal neuronal activity. The trisomy 16 mouse is considered to be a model of human trisomy 21 (Down Syndrome). It has been reported that both low voltage activated (LVA) and high voltage activated (HVA) calcium currents are present in embryonic hippocampal neurons in primary culture. Hippocampal neurons were cultured from fetal trisomy 16 and normal mice at gestation day 14-16 using standard techniques. Whole cell inward currents were recorded from the somatic region of 10-21 day old cells, with 120 mM choline chloride, 20 mM 4AP, 10 mM CaCl2, and 10 mM TEA in the bath solution. Currents were activated by 10 mV, 150 ms steps, from holding potentials of -90, -40, and -60 mV. Both HVA and LVA calcium currents were identified. In trisomy neurons the peak amplitudes of the inward current reached a maximum at a membrane potential (Vm) of 6.7 mV (n = 12) for currents evoked from a holding potential of -40 mV and at 0.4 mV (n = 15) for currents evoked from a holding potential of -90 mV. The latter currents showed a statistically significant hyperpolarizing shift for Vm of 7.5 mV compared to control neurons. During the recording time the I-V response of the trisomy 16 cells showed a significant shift of 7.1 mV (n = 15) in a depolarizing direction which was not observed in control neurons. These observations suggest that calcium currents are abnormal in trisomy 16 fetal hippocampal neurons.
450.3 ACTION POTENTIALS IN CULTURED FETAL HIPPOCAMPAL NEURONS FROM MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. N.N. Con, Z. Calafatck & S.L. Rapaport. LNS, NIA, NIH, Bethesda, MD 20892.

The trisomy 16 (Ts16) mouse is considered to be a model of human trisomy 21 (Down syndrome). This laboratory has reported that action potentials in Ts16 and Ts21 cultured dorsal root ganglion (DRG) neurons are different from those in control neurons. The aim of these experiments was to investigate action potentials in Ts16 hippocampal neurons. Primary cultures of hippocampal neurons were prepared from Ts16 embryos and littermate controls at gestation day 14-16, whole cell patch clamp recordings were made from cells of the CA1 region as well as in the more superficial cortical layers. Cortex slices placed with the pial surface and show dense fiber processes are visible growing into the adjacent cortex slice. Many labeled axons gave rise to AChE-positive axons. ChAT-immunostaining reveals positively labelled neurons only in the basal forebrain tissue. Within one week of culturing, AChE-stained fiber potentials were evoked by 0.1 to 0.8 nA depolarizing pulses. All measurements were done at -60 mV. Data were taken from 10 control and 14 Ts16 cells. Resting membrane potentials were -49 mV and -42 mV for control and Ts16 neurons respectively. In control cells the mean action potential amplitude ranged from 87 mV (0.1 nA pulse) to 154 mV (0.8 nA pulse), the mean time to peak amplitude from 5.3 to 3.9 ms, the mean width from 3.5 to 3.6 ms, the mean depolarization rate from 100 to 117 V/s and the mean repolarization rate from -51 to -54 V/s. None of the mean passive or active membrane properties measured in Ts16 neurons was significantly different from control values. This result is different to that reported in Ts16 and Ts21 DRG neurons, and might be explained in differences in maturation and/or the presence of growth factors.

450.5 CHOLINERGIC RESPONSES OF DEVELOPING RAT NEOCORTICAL CELLS IN TISSUE SLICES, A LAMINAR STUDY, Finlayson, P.G. and Cynder, M.S., Dept. of Ophthalmology, Univ. of British Columbia, B.C., V5Z-1N9.

Enhanced plasticity of neonatal systems are paralleled by developmental changes in the cortical laminar distribution of many receptors, as observed through pharmacological studies. Muscarinic cholinergic receptor subtypes, M1 and M2, are distributed in the developing rodent and kitten cortex, and are transiently high in layer IV of young ages but predominantly concentrated in layers I, II and III in adult cats. Nicotinic receptor mRNA is abundant in layer IV of rat pial visual and auditory cortices during the second postnatal week. In this study, physiological correlates of receptor redistributions during development were examined. Responses of neurons in layers 2/3, 4, and 5 in the visual and non-visual cortex region were recorded using a micromanipulation technique. No evidence for specific receptors was observed at any developmental stage. The development of these responses was correlated with changes in the laminar distribution of cholinergic and serotonergic axonal innervation in the developing cortex. The role of afferents in cortical neuron development is being studied in the mouse thalamocortical slice model. Neurotransmitters may act to facilitate examination of cellular mechanisms that underlie postnatal development and the factors that may influence cortical development. This work was supported by grants from Alzheimers Fed. 90-082, NS2674 (RTK) and NS27501 (DO).

Co-cultures of hippocampal (HC) and locus coeruleus (LC) were studied immunocytochemically to examine noradrenergic innervation of hippocampus. Vibriomce slices (350 μm) of P4 rat HC and P17-21 LC were co-cultured on glass coverslips for 2-8 weeks. TH and DBH-positive cells were then evaluated in cultures and cells. Large, multipolar cells were labelled in LC, and an occasional cell appeared near or within HC in and fibres from DBH-positive cells tended to converge at the edges of LC tissue, apparently having migrated out of this region. LC slices cultured alone showed little sign of fibre outgrowth in any direction. TH- and DBH-positive fibre tracts were traced from LC to HC. Cresyl violet counterstaining used to distinguish hippocampal cell layers, revealed that immunopositive fibres entered the HC at the hilar and CA3 regions. Electron microscopic studies are currently underway to determine the postsynaptic targets of these fibres. Supported by NIH grants NS15317, NS18895, NS08639.


Intracellular recordings and labelling techniques have become extremely important in the physiological and morphological identification of neurons in the central nervous system. Intracellular dyes, such as biocytin/Neurobiotin (BC/NCB) and Lucifer Yellow (LY), have been used for the morphological and electrophysiological characterization of neural circuits in vivo and in vitro. To improve on the morphological characterization of single neurons in the rat hippocampus, investigators have combined use of biocytin/Neurobiotin (BC/NCB) and LY with electron microscopy. The combination of these two techniques, however, is not without problems. For example, LY does not penetrate into the dendrites of the pyramidal neurons. 

Supported by NIH grants NS18955 and NS20482.

As part of our continuing comparison between normal human developmental patterns and changes seen in neurodegenerative disease, we examined the distribution of diaphragm (NADPH-D)-containing neurons within the medial temporal lobe of the embryonic human brains at gestational (E) points 10-26 weeks. No NADPH-D-containing profiles appeared in the medial temporal lobe prior to week E19. At E19, faint NADPH-D staining was seen within the central nucleus of the amygdala. Staining for Nissl substance and acetylcholinesterase (AChE) at E19 revealed an immature, but existing, cytoarchitecture suggesting that the neurons which normally synthesize NADPH-D are present but are not expressing the enzyme. At E21, the accessory basal and lateral basal amygdaloid nuclei contained numerous NADPH-D-positive neurons and a dense neuropil. The lateral amygdaloid nucleus was moderately labeled. The entorhinal cortex displayed a trilaminar pattern with darkly labeled cell islands in layers I-II, a dense neuropil in B-III and V-VI. In the hippocampus, a continuous band of NADPH-D was seen from CA1-CA3. CA4 contained only light NADPH-D staining. NADPH-D-stained profiles were also seen in the dentate granule cell layer. Clouds of NADPH-D stained neuropil were seen in layer II of the subiculum. At E24-26, NADPH staining within the medial temporal lobe resembled the adult. These data will be compared to potential morphological changes seen in normal aging and disease.


Three novel Antp.-class homeobox genes have been isolated from a human 11 wk. fetal brain cDNA library. PCR with two sets of oligonucleotide primers (specific for highly conserved regions of the Antp.-class homeobox) was used to amplify portions of homeobox genes present in the fetal brain library. The products were cloned into a modified pGEM vector and transformed into DH5a cells. Sequencing 100 clones (spanning 77 to 81 bases between primers) identified 11 unique Antp.-class genes. The base sequence of one of the novel HOX genes, HOXBA, showed 65% homology with HOX1-1, 8% homology with the CHOX7 gene (isolated from fetal chicken), and 95% homology with the MMXA gene (isolated from mouse telencephalon). Based on the origin of MMXA, it appears that HOXBA is among the first Antp.-class homeobox genes in the developing human telencephalon.


The goldfish visual pathway displays continuous growth, development, and plasticity throughout its life. Furthermore, a remarkable capability for functional regeneration occurs after optic nerve injury. This laboratory is interested in intermediate filament (IF) proteins and their structural relationship to cellular growth and development. Glial cells in this pathway express a type II keratin, not the expected GFAP. The presence of a type II keratin predicts the expression of a type I keratin partner. Using PCR, a cDNA encoding a keratin was amplified from a goldfish optic nerve cDNA library. DNA fragments from this core region were then used to rescreen the cDNA library to isolate larger clones. The largest cDNA clone was translated, compared to known IFs, and classified as a type I keratin. Northern analysis revealed an mRNA of 1.6 kb that is abundantly expressed in the optic nerve. In situ hybridization on regenerating optic nerve shows that both the type I and type II keratins have increased mRNA levels throughout the optic nerve 10 days after nerve crush.

Keratins are known to be expressed in mammalian CNS only in early development. Thus, these IF proteins may have structural attributes which support the growth characteristics of this pathway and may be linked to the capacity for growth in this system. (Supported by NIH grant EY05212 to NS).


Optic axons undergo a transformation in their fiber ordering within the chiasmatic region to become segregated by type within the optic tracts. In the tract, these segregated axon classes are themselves roughly retinotopically ordered, particularly with respect to the dorso-ventral retinal axis. The present study has examined the emergence of this retinotopy within the chiasmatic region of adult ferrets and cats. Ferrets were given discrete retinal lesions and were then examined for the distribution of degenerating myelin in semi-thin sections along the course of the fiber pathway. Cats were given implants of Horseshadish peroxidase via a transbuccal approach to label the chiasmatic path and retinal origin of the superficially placed axon classes counting through the lateral or medial halves of the optic tract.

The topographic ordering of individual axon classes observed in the tract must be established afresh within the chiasmatic region, since 1) axonal degeneration induced by dorsal vs. ventral retinal lesions is relatively well segregated in the pathway, and 2) degenerating myelin is widely dispersed along the intracranial and pre-chiasmatic segments of the nerve in adult ferrets. In both the dorsal and ventral tracts, axons are intermingled in the pre-chiasmatic optic nerve of adult cats derived from the ventral or dorsal retina. Dorsal retinal axons destined to enter the contralateral optic nerve are segregated into dorsal or ventral classes, prior to crossing the midline they are intermingled. The degree of retinotopic precision becomes too degraded along the nerve to account for the order found in the optic tract. An active re-organization must occur within the chiasmatic region, and for the degenerating fibers this occurs in the region of the midline.
541.3

THE ARRANGEMENT OF AXONS BY SIZE AND LATERALITY OF PROJECTION IN THE OPOSSUM’S OPTIC TRACT.

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We have determined the relative positions and size spectra of crossed and uncrossed optic axons in the opossum’s optic tract by comparing their organization with both the patterns of ganglion cell geneline and the disposition of axonal terminations in target nuclei. Results of axon size show that (1) the deep parts of the OT contain medium and fine fibers with uncrossed axons displaced pia marginis, (2) the intermediate parts contain axons of all diameter classes with coarse crossed elements deeper than uncrossed counterparts, (3) the superficial parts contain primarily crossed, fine fibers and mostly uncrossed, medium and coarse axons. Thus, as in eutherians, the fiber order in the opossum’s OT arises from the sequences of genesis of ganglion cells and the preservation of the pathway present early in development. Johnson, et al. (1990, Soc. Neurosci. Abstr., 16:53) also reported similar transcellular diffusion events of carbocyanine dye, DiA, in the embryonic ferret visual system. Kelly, Johnson, and Casagrande, 13 Dept. of Cell Biology and Psychology, Vanderbilt University, Nashville, TN.

541.5


Optic axons appear to make a choice at the optic chiasm. It is unclear what mechanisms may signal such a choice. We inoculated DiA into embryonic eyes between E21-E30 to examine interactions between crossed/uncrossed labeled retinal axons at the optic chiasm. Embryos were delivered via cesarean section from anesthetized ferrets and immersion fixed in 4% paraformaldehyde in phosphate buffer. DiA crystals (4-[dihexadecylamino]styryl)-N-methylpyridinium iodide; Molecular Probes) were then put into the eyecup. Following incubation at 37°C for 1-2 months, brains were removed and serial sectioned with a cryostat. Nodes of Ranvier and myelin sheaths were identified by anti-galactocerebroside and anti-myelin basic protein antibodies. We found that the DiA+ population was always in the fusion area of the eye fragment, i.e. in the temporal part of the retina. The cut edges enclosed the lens and fused together in the temporal region of the orbit, with this fusion occurring within 3-5 days. Computer reconstructions of serial sections show that the cut edges become stikingly labeled beginning at this age. Ganglion cells and radial glial cells, putative Müller cells, also become labeled. Transcellular labeling of thalamic radial glia bordering labeled optic tracts was routinely observed. Transneuronal diffusion into cortex was not observed. These results suggest that the dye communicates between cells (neuron-neuron, neuron-glia) at and near the optic chiasm, and within the retina, via specialized cell-cell contacts. Alternatively, the strongly labeled projection to the contralateral retina may reflect an interretinal pathway present early in development. Johnson, et al. (1990, Soc. Neurosci. Abstr., 16:53) reported similar transcellular diffusion events of carbocyanine dyes in fixed tissues of the horseshoe crab. Supported by grant EYO0835 (VAC), core grants EYO0812 & HD15002.

541.7


In Xenopus embryos, the formation of the early eye is guided by a series of morphogenetic events (Frank and Schwab, 1989). The retinal axons extend from the eye via the first and second eye nerves, which innervate the Bruchner's nodules and the optic chiasm, respectively. They establish transient connections with the tectum and tectobulbar system. The first wave of retinal axons is followed by a second wave, which extends further across the entire retina, some with well-developed dendrites. SEM observations suggest that retinal positional information is present for the decussation of the early projection cohort, but that expression of this information is dependent upon interaction of naso and temporal axons at the chiasm. Nasal axons alone may not be endowed with explicit instruction to cross the chiasm. Temporal axons seem to avoid a sector of the midline chiasm and grow along crossed fibers from the other eye (Godement, Salavin & Mason, 1990). Additionally, prior to their crossing of the chiasm, nasal axons may avoid growing along the temporal axons filling the ipsilateral optic tract. A guidance mechanisms for crossed projection from the nasal retina would not be operative until the arrival of temporal axons at the chiasm. (Supported by NIH R01 19245).

541.4


Using DiI in fixed embryos of the rhesus macaque (Macaca mulatta), the first optic axons are seen to enter the optic tracts by embryonic stage 21 (E21). At stage 21 (E42), reticuloglial labeling of the optic tract reveals that the most advanced axons arise from ganglion cells within a discrete area just dorsal to the optic nerve head and far from the prospective fovea. Given the exceptional hemiretinal specificity evident in later fetal ages (Chalupa & Lii, 1991), it is remarkable that the first ipsilateral projections arise from this portion of the presumptive nasal hemiretina, similar to the case in the mouse (Collier & Guillery, 1980). By the end of the embryonic period (E42), the most advanced projections arise from scattered ganglion cells distributed in relatively low density across the entire retina, some with well-developed dendrites. Early projection cohort exhibits the inherent primate decussation pattern, lacking the crossed temporal projection present among nonprimate mammals.

541.6


After early postnatal lesions in the hamster, the retinal convergence from the retina to the tectum increases at the population level but is unchanged at the single neuron level, i.e., the visual receptive fields of single neurons are the same size as normal (Pallas and Finlay, 1989). Thus, how developing connections are adjusted in order to meet both functional needs is of interest. In this study, we used an in vivo technique to follow the growth and development of axonal arbors in both normal and early partial tectectic. A 3-day-old retinal half was placed into the brefuxin, the brain was incubated in a culture media for the transport of HRP (14 or 4 hours) and then processed with immunocytochemistry. Xiong arbor were branched and branching pattern and bouton number were quantified for each side. Axon arbors (n=10) from lesioned tecta were qualitatively less complex: covered a smaller area, had fewer branches, and had significantly decreased bouton numbers compared to the normal (N=7) (Normal=113±17, Lesion=63±9; t=4.95, p<.001). These findings indicate that each retinal axon makes fewer contacts in the lesioned tectum than normal to compensate for the numerical disparity between number of retinal axons and target size.

Since prior research has shown that cell death is little changed and synaptic density is unchanged in this preparation, present data indicates the principal means of adjustment of numerically disparate populations is alteration of axonal arborizations. A more extensive morphological examination is under way to describe in more detail the structural changes in axonal arborizations. Supported by NIH R01 NS 19245.

541.8

ANTIBODIES AGAINST NEURITE GROWTH INHIBITORS FROM OLGODENDROCYTES CHANGE THE COLICULAR TERMINATION PATTERN OF POSTNATALLY SPROUTING RETINAL FIBERS. Joseph P. Carmack1 and Gerald E. Schneider2.


After early postnatal unilateral ablation of the superior colliculus (SC) together with the ipsilateral eye in Syrian hamsters, retinal fibers abnormally cross the tectal midline and innervate the remaining SC (Schneider, 1978). The recrossing retinal fibers are mainly confined to the stratum griseum superficiale (SGS), with little ingrowth or termination in the deeper stratum opticum (SO); lately, most termination is in the superficial part of SGS. Establishment of this pattern, observed at postnatal day 12, is temporally correlated with the appearance of oligodendrocytes in the S0 during growth of recrossing fibers (Schneider et al., SN Abstr., 90; Carmack et al., SN Abstr., B7).

We now show that application of the IN-1 antibody directed against neurite growth inhibitors from oligodendrocytes (Caron and Schwab, 1988) changes this termination pattern. In the presence of this neutralizing antibody, but not with a control antibody, recrossing retinal fibers traverse the SO as well as the SGS, with greater depth of termination in SGS and SO. This pattern resembles that of the normal contralateral retinotectal projection.

The results indicate that neurite growth inhibitors expressed by oligodendrocytes are responsible for the innervation of a target area in postnatal plasticity. Supported by NIH grant EYO0136, Swiss National Science Foundation, EMBO.

VIP and serotonin receptors mediate the topographic mapping of retinal ganglion cell terminals onto the optic tectum of the frog (Cline & Constantine-Paton, Neuron, 3:413-426, 1989). In other systems, VIP has been shown to modulate the activity of NDMA receptors (Reynolds et al., Brain Res. 456: 28-292, 1988; Sah, Biophys. J. 53:356a, 1988). We wish to determine if serotonin and VIP are involved in topographic mapping of synapses by interacting with the NDMA receptor. As a first step we have investigated the distribution of these two substances in the frog visual system using immunocytochemical techniques. VIP-like immunoreactivity was detected in both the cellular and plexiform layers of the optic tectum. VIP-like immunoreactive (VIP-IR) cell bodies constituted 19, 26 and 29% of the cells in layers II, IV and VI, respectively. In addition to being located in layers V and VII, VIP-IR fibers were also found in layer IX where retinal ganglion cells terminate. The nucleus internum (NI), a structure reciprocally connected to the optic tectum, also contained a high proportion of VIP-IR cells in the posterior tectal cortex (53%). No VIP-IR cells were found in layer I. There were very few VIP-IR cell bodies in the anterior nonrim cortex. The thalamus, which has several projections to the optic tectum, also contained many VIP-IR cells. Serotonin-like immunoreactivity in visual areas was limited to a subset of cells in tectal layer VI and a ventral thalamic region. This project was supported in part by BRSG S07-RO1T14-22.

51.11 QUANTITATIVE ANALYSIS OF AGONIST-EVOKED CURRENTS IN IDENTIFIED TECTAL NEURONS OF RANA PIPIENS. E.W. Hickmott and M. Constantine-Paton. Dept of Biology, Yale University, New Haven, CT 06511.

Previous work in our lab has implicated the N-methyl-D-aspartate (NDMA) receptor in the activity-dependent refinement of the retinotectal map in Rana pipiens. For example, NDMA treatments of the axon terminal of the retina-tectum projection causes both a sharpening of the retinotopic map, and a decrease in the sensitivity of the tectum to acutely applied NDMA (E. Debabi and M. Constantine-Paton, 1990). However, it is not known whether this decrease has only been examined at the level of the electrically-evoked field potential, the precise mechanism of this functional decrease is unknown. To address this question, we have developed a tectal slice preparation in which we can record from single tectal neurons, using whole-cell voltage clamp. Previously, we have examined postsynaptic currents evoked by electrical stimulation in the optic tract, and the effects of bath application of various drugs on these PSICS (P. Hickmott and M. Constantine-Paton, Abstr, 1990).

Instead of using electrical stimulation to evoke the PSICS, we have now developed techniques for using iontophoresis of neurotransmitter agonists to evoke PSICS. We have determined that tectal neurons can respond to iontophoresed glutamate, NDMA, AMPA, and GABA; in some cases neurons respond to all these agonists. By using multibarreled iontophoreses we will be able to determine the quantitative relationship between these currents and various morphologically-identified cells in the tectum. Thus, we will be able to examine the question of whether certain tectal cell types respond preferentially to one agonist over another, which will give us further insight into the tectal circuitry. We will compare these data from normal tadpoles to similar data obtained from tadpoles that have been treated with AP5, MK801, or MK801 + NMDA, to determine whether these alternations are accompanied by changes in retinotopic synaptic contact we have examined the distribution of synapses within single HRP-filled, RGC terminal arbors in NDMA-treated tecta. The tecta of young post-metamorphic frogs were treated with a low dose of NDMA for 4 to 5 weeks and then processed for EM. The morphology of the HRP-Filled, IR-Peroxidase labeled tectal terminals were then reconstructed at the light microscope level (LM). Using the centroidal Strehler scheme we labeled all the branching segments on the RGC terminal giving a hierarchy of order 5 to 1. Order 1 references the most distal branches and order 5 to the major axon cylinder. The ratio of synaptic contact to axon diameter was calculated for the EM level, and multiplied by the total axonal length measured at the LM level to estimate total synaptic contact for each branch order. These values were normalized to give the percentage of total synaptic contact contributed by each branch order within the arbor.

The analyses showed that, in the NDMA-treated arbor, the distal order 1, 2, 3 branches contained almost all the synaptic contact, 42%, 35%, 17% respectively. This synaptic distribution is similar to that of Type IV arbor in normal tecta even though NDMA-treated arbor are less branched. The decrease in branching and the maintenance of the same proportion of total synaptic contact contributed by each branch order suggests a proximal shift of synaptic distribution toward the main axon in NDMA-treated arbor. In addition, these analyses suggest that total synaptic contact of NDMA-treated arbor decrease relative to that of the arbor in normal tecta. However, this may not indicate a decrease in total synaptic efficacy: Earlier analyses (Yen & Constantine-Paton, 1990 Proc. Soc. Neurosci 16: 532.14) showed an increased synaptic convergence and thickened pre- and postsynaptic specializations of the NDMA-affected tecta. (Supported by NIH grant EY07860).
451.15

Neurons of the posterior thalamus (PCL) that project to the LPN in hamsters can be divided into distinct morphological classes. Neonatal enucleation has only slight effects upon the morphology of cells within a given class, but significantly reduces the number of neurons (small and widefield vertical) with dorsally directed dendrites. Does this change reflect differential transneuronal degeneration or alterations in the dendritic arbors of surviving cells in this study, we restricted our analysis to a specific and relatively homogeneous subpopulation of superior collateral neurons, those that project to the lateral posterior nucleus (LPN). The combination of retinotopic organization and intracranial injection of Lucifer yellow demonstrated that over 75% of tecto-LP cells in normal hamsters are widefield vertical cells. Most tecto-LP cells in the neonatally enucleated hamsters were either horizontal cells (17%), giant stellate cells (25.5%), or had dendrites that were directed toward the deep SC laminae (10.1%); less than 25% were widefield vertical cells. Differential enucleation-induced cell death could not account for all of these changes. Tecto-LP neurons were retrogradely labeled with DiI in hamsters on postnatal day 0 to 10. As early as P0, most retrogradely labeled neurons could be identified as either widefield (44%) or narrowfield (18.9%) vertical cells. These results support the conclusion that neonatal eye removal results in a reorganization of dendritic arbors that have already undergone considerable development at the time of the lesion. EV 04170, EV 08015.

451.17

We have employed immunocytochemistry in conjunction with both light and electron microscopy to describe the normal development of the serotoninergic innervation of the hamster's SC. Serotoninergic-immunoreactive fibers in adult hamsters are present in all SC laminae and are most dense in the lower part of the stratum primate superficiale (SSS) and stratum opticum (SO). Serotoninergic-immunoreactive fibers were visible in the SC by embryonic day 12 (two days prior to birth). By the day of birth, some immunoreactive fibers were present just below the pial surface and other were generally oriented either parallel or orthogonal to the SC laminae. Over the next two weeks, the serotonergic innervation of the SC increased in density and assumed the laminar distribution observed in adult hamsters. Electron microscopic examination of 113 5-HT-positive varicosities in single thin sections taken from the SSS and SO of adult hamsters revealed only 5 conventional synaptic contacts while analysis of immunofluorescent varicosities from hamsters killed on postnatal day 3 revealed 103 conventional synaptic contacts, most of which were axodendritic. Serial section analysis of additional 5-HT-positive swellings from adult hamsters provided further support for the conclusion that very few serotoninergic axons make synapses in the SC of these animals. These results indicate a substantial synaptic reorganization of the serotoninergic input to the SC in developing hamster. The role that these transient synapses might play in collicular function or development is currently unknown. EV 04170, EV 08015.

451.19

To further define the anatomical reorganization associated with the cortical-retinal projection of the hamster's SC. Serotoninergic-immunoreactive fibers in adult hamsters are present in all SC laminae and are most dense in the lower part of the stratum primate superficiale (SSS) and stratum opticum (SO). Serotoninergic-immunoreactive fibers were visible in the SC by embryonic day 12 (two days prior to birth). By the day of birth, some immunoreactive fibers were present just below the pial surface and other were generally oriented either parallel or orthogonal to the SC laminae. Over the next two weeks, the serotonergic innervation of the SC increased in density and assumed the laminar distribution observed in adult hamsters. Electron microscopic examination of 113 5-HT-positive varicosities in single thin sections taken from the SSS and SO of adult hamsters revealed only 5 conventional synaptic contacts while analysis of immunofluorescent varicosities from hamsters killed on postnatal day 3 revealed 103 conventional synaptic contacts, most of which were axodendritic. Serial section analysis of additional 5-HT-positive swellings from adult hamsters provided further support for the conclusion that very few serotoninergic axons make synapses in the SC of these animals. These results indicate a substantial synaptic reorganization of the serotoninergic input to the SC in developing hamster. The role that these transient synapses might play in collicular function or development is currently unknown. EV 04170, EV 08015.

451.16

It has been well-documented that neonatal enucleation causes marked changes in the number and function of cells located in the superficial layers of the superior colliculus (Findlay et al., Dev. Brain Res. 28: 1, '86, Rhoades, J. Physiol. (London) 301:383, 300; Tokunaga et al., Brain Res. 528: 467, '90). How this deafferentation effects specific populations of neurons in these layers has not been well defined. Adenosine deaminase immunoreactivity (ADA-IR) can be used as a marker for a specific and relatively homogeneous subpopulation of superior collateral neurons, those that project to the lateral posterior nucleus (LPN) of the thalamus (Miguel-Hidalgo et al., Brain Res. 476:183, '89). We undertook this study to examine the effects of fetal and neonatal deafferentation on the ADA-IR population of SO cells. Unilateral and bilateral enucleations were performed on rats at embryonic day 15 or at birth. All animals received injections of fluorescein labeled beads into the SC. In normal animals, there was an average of 718 ± 56 S.D. ADA-IR SO neurons per colliculus of which 498 ± 228 ADA-IR cells projected to the LPN. Surprisingly, neither the fetal enucleations or the neonatal enucleations had an effect upon the total number of ADA-IR SO peripherally deafferented (1927 ± 600 and 2068 ± 70, respectively) or the number that projected to LPN (677 ± 326 and 576 ± 28, respectively). These results indicate that a specific population of neurons in the superior colliculus is refractory to neonatal or fetal deafferentation. Support: EY04170, EY08015, HL36575.

451.18
CORTICAL FEEDBACK TO LGN MAY PLAY A MAJOR ROLE IN OCULAR DOMINANCE COLUMN DEVELOPMENT. K.P. Umphres and Harton S. Nizic, GM Research Labs, Warren, MI 48090 and University of Michigan, Ann Arbor, MI 48109.

A model is presented to investigate the role of feedback in the development of ocular dominance columns in the primary visual cortex. In our model, summed response of cortical neurons is fed back to the LGN to modify their activities and consequently their connection strengths to the cortex. The synaptic modifications in the LGN are used to establish connections between neurons in local LGN circuits and the cortical feedback signals and can easily be carried out by neuronal circuitry containing NMDA-type receptors.3 It has been observed that during development, the subplate neurons, situated just below the cortex, project axons to the thalamus and dendrites to the deep SC layers.2 This provides a mechanism for sending summed cortical responses to the geniculo-cortical neuron. Recent evidence suggests that the death of the subplate neurons5 and inactivation of the NMDA receptors6 coincide with the termination of ocular dominance column development also supports our model.


451.20
TEMPORAL-FREQUENCY SENSITIVITY OF LATERAL GENICULATE NEURONS IN AGING RHEUS MONKEYS. Charles B. Y. Kim*, Rodney J. Moore, Peter D. Spear, Risa Tumino, and Jin-Tang Xue*. Department of Psychology and Center for Neuroscience, Univ. of Wisconsin, Madison, WI and School of Ophthomology, Univ. of Missouri, St. Louis, MO.

Human psychophysical studies have demonstrated decrements in temporal-frequency sensitivity as a function of aging, even after pre-retinal ocular media differences have been controlled for experimentally. These studies have suggested either retinal and/or post-retinal (central visual pathways) sources for the differences in performance. In an effort to understand the nature and origin of the modifications of the visual pathways during aging, we recorded extracellularly from single neurons in the parvocellular (PC) and magnocellular (MCL) layers of the lateral geniculate nucleus (LGN) of young (5-16 yr) and old (25-28 yr) rhesus (Macaca mulatta) monkeys. Each neuron was stimulated with gratings drifted at 10 different temporal frequencies (1-30 Hz) at its most optimal spatial frequency and a sub-saturation contrast level. Optimal temporal frequency, bandwidth, and high and low temporal frequency cutoff values were determined for each. Neuronal receptive fields were matched for eccentricity (0.15 deg). Our preliminary results indicate that aging has no significant effect on temporal-frequency sensitivity of PC or MCL neurons in the monkey LGN. This suggests that age-related neural deficits in visual temporal function may exist in more central visual structures, such as striate cortex. (Supported by EY01916, EY02945, and F32 AG05526.)
451.21

The monocular antibody ABS, which is specific for ganglion cells in the retina of cat and rabbit, also stains a limited subset of neurons in central nervous system. In lateral geniculate nucleus (LGN), it stains some of the larger neurons. We here report, that in monocularly deprived cats, ABS immunoreactivity is decreased in the deprived laminae. These long-term monocularly deprived cats were deeply anesthetized with i.v. Nembutal and perfused through the heart with buffered saline followed by 4% carbodiimide, 4% paraformaldehyde and 4% sucrose in 0.1 M phosphate buffer. Thalamos was frozen and sectioned coronally at 30 μm into 0.1 M phosphate with 0.02% azide. Sections were then immunoreacted for the presence of ABS using standard ABC protocols. Although the staining of the retinal terminals did not appear to be affected by the monocular deprivation, staining of cell bodies was reduced in the deprived layers. These findings are reminiscent of those obtained with the monocular antibody CAT-301 (Sur et al. J. Neurosci., 8:274, 1988), and suggest that the ABS staining is associated with cells that are particularly vulnerable to abnormal early experience, perhaps Y-cells. (Supported by NSF grant BNS 8811039 to SBT and PHS grant EY 06496 to KRF.)

451.22
NGF PREVENTS THE SHRINKAGE OF NEURONS IN LATERAL GENICULATE NUCLEUS (LGN) OF MONOCULARLY DEPRIVED RATS. L. Domenici, A. Gellerino * and L. Maffei *.

Istituto di Neurofisiologia (CNR) and Scuola Normale Superiore, Pisa, 56100, Italy.

In cats and monkeys monocular deprivation (MD) causes the shrinkage of neuronal perikarya in the deprived laminae of LGN, in correspondence with the binocular visual field. Here we report that the NGF supply prevents this shrinkage in the rat. To identify the ipsilateral LGN laminae which project to the binocular portion of the rat visual cortex a HRP-solution (10 μl, 30% HRP plus 2% DMSO in saline) was injected into the deprived eye and after a 24-36 hours survival time the brain was fixed, processed by standard HRP histochemistry and Nissl method. One month of MD causes a shrinkage in the LGN deprived lamina with respect to the undeprived lamina (mean difference in some size = 22%). By contrast, the soma size distributions for the deprived and undeprived LGN laminae extensively overlap in MD rats treated with NGF.

We conclude that NGF treatment rescues from atrophy the neurons in the LGN deprived lamina projecting to the binocular portion of the visual cortex.

451.23

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During development, orderly projections from the retina to the lateral geniculate nucleus (LGN) are established through remodeling of a more diffuse set of immature projections. Neuronal activity is thought to play an important role in the remodeling process (Shatz and Stryker; 78; Hahn et al., 91). We have begun to examine ferret LGN neurons in slices to determine the degree of maturation of membrane and synaptic properties during a period that retinogeniculate afferents undergo considerable change. In the ferret, segregation of retinogeniculate afferents into eye-specific layers is essentially complete by postnatal day 14 (P14) and is more obvious by P21 (Linden et al., 91; Hahn et al., 91). In vitro intracellular recordings were carried out in animals from P1 to P33. Resting membrane potentials (range 51-67 mV), input resistances (26-118 Mohms) and time constants (5-11 msec) of the LGN cells showed no clear changes with age. At all ages, depolarizing current injection could elicit action potentials. The first low-threshold spikes, common in the adult LGN, were observed at P9 and were encountered routinely thereafter. Also at P9, the first postsynaptic potentials evoked by stimulation of the optic tract were recorded. In one neuron at P9, optic tract stimulation evoked an IPSP with a reversal potential of -56 mV. At later ages epsps were more commonly observed. Our data indicate that at least some cells within the LGN are electrically excitable and make functional contacts with retinal ganglion cell axons during the period of segregation of retinogeniculate afferents. Supported by EY 06297 (C.A.W.) and EY 07023 (M.S.).

451.24
NMDA ANTAGONIST INFUSION DURING ON/OFF SUBLAMINAR SEGREGATION ALTERS DENDRITIC MORPHOLOGY OF CELLS IN FERRET LGN. M. Rakesh and M. Sur.

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The NMDA subtype of glutamate receptors may contribute to the establishment of specific connections in the developing central nervous system. Recently, chronic infusion of the NMDA antagonist, D-APV, has been shown to affect sublaminar segregation in the ferret LGN (Linden et al., 91). Altering the morphology of retinogeniculate afferents (Hahn et al., 1991). Here, we examine whether NMDA receptor blockade also affects the morphology of the postsynaptic target cells in the LGN.

The specific NMDA antagonist D-APV (0.8 mM) was chronically infused via an osmotic minipump into the thalamus of ferrets for one week from P14 to P21 (n=4) when retinogeniculate afferents normally segregate into On and Off sublaminae. Rhodamine-labeled latex microspheres were injected into visual cortex (area 17) to backfill and identify LGN relay cells. Immediately after treatment, these cells were intracellularly injected with Lucifer Yellow in living 300 μm horizontal slices.

D-APV treatment led to an increase in dendritic complexity of LGN cells, shown by an increased number of branch points (from 23±11 in normal animals at P21 to 60±128 in treated animals, p<0.001). Dendritic arbor area also increased from 26826±16626 μm² in the normal group (26 cells, 3 animals) to 48831±43867 μm² in the treated animals (19 cells, 5 animals, p<0.01). These effects were particularly marked in the A laminae. Some sizes were unaffected by NMDA receptor blockade.

These results indicate that the dendritic development of LGN cells is markedly affected by NMDA receptor blockade, and suggest that synaptic transmission through NMDA receptors may play a significant role in the concurrent maturation of postsynaptic afferents and postsynaptic target cells.

Supported by EY07003 and CNPq.
The effect of cyclosporine-A on eye removal-induced rejection of mesencephalic retinal transplants. Ghost cell formation in the retina was monitored with the aid of confocal microscopy. The results indicated that cyclosporine-A (CyA) significantly delayed the onset of the immune rejection response.

In order to achieve our ultimate goal of restoring vision to blinded eyes by use of retinal transplants, we had to develop a technique for the long-term survival of retinal grafts in the host eye. This required a better understanding of the immune rejection process. We used a combination of immunosuppressive drugs, including cyclosporine-A, to prevent the rejection of retinal transplants.

The study reached its conclusion by comparing the survival of retinal grafts in the presence and absence of various immunosuppressive drugs. It was found that cyclosporine-A was highly effective in prolonging the survival of retinal transplants, and that its use should be considered for clinical applications.

545.2 INTRARETINAL XENOGRAFTS OF MITOTICALLY ARRESTED HUMAN RETINOBLASTOMA CELLS. E. S. Lazar, M. del Cerro, M. L. Agostini, and M. del Cerro, Department of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, New York, 14642, USA.

Retinoblastoma is the most common ophthalmic malignancy in childhood. Cell lines obtained from these tumors have been the topic of numerous studies at both the cellular and molecular levels. This project was supported by the use of one of these cell lines (Y 79) for intraretinal xenografts into the mesencephalic retina, in an attempt to determine the survival, integration, and differentiation characteristics of these cells after they had been transplanted into the eye of a healthy host.

In conclusion, retinal transplants derived from cell aggregates contain immunogenic disparity between graft donor and host, and implants into a site that is not hospitable to retinal implants, presumably because the eventual success of these grafts requires appropriate growth and differentiation factors and opportunities for relevant neural connections. This successful engagement of developing retinal tissue into the eye can be expected to depend upon (a) immunogenic disparity between graft donor and host, and (b) implantation into a site that is not hospitable to retinal implants.
452.7

In normal rats, as in humans, a light flash inhibits an acoustic startle reflex elicited briefly afterwards, with the degree of inhibition determined by the intensity of the light as well as by the test interval. We will demonstrate that the initial effect of light damage to the retina in the albino rat slows the rate at which peak inhibition develops (the standard 30-40% flash has its peak at about 70 ms, which shifts by about 100 ms with 48 hour exposure to 300 ft-c). Decrements in the strength of inhibition are seen only with longer exposure times. The same temporal shift precedes the loss of inhibition in RGCs rats, in which photoreceptor damage occurs because of a degenerative disease. We show further that the isolated temporal shift in peak inhibition seen with early retinal damage cannot be simulated in normal rats, but by lowering the stimulus intensity, the stimulus duration, or the degree of light-adaptation. This data reveals that these early disease states cannot be modeled solely by decrements in sensitivity or integration time, or by dark-adapting mechanisms. We can conclude from this study that early retinal damage directly revers the speed of sensory processing. (Supported by NIMH grants 5T32MH007062, EY-01319, EY-0243, and generous private donations.)

452.9
Department of Ophthalmology, Tokoku University School of Medicine, Sendai Japan*; Department of Neurobiology and Anatomy**, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC, USA.

Age related retinal cell death in macular degenerations may in part be due to an aging RPE which loses its capacity to support photoreceptor cells. We wished to test the hypothesis of delaying photoreceptor cell death in the aging, normal retina by introducing young RPE into older eyes of an appropriate model. We have used the Fischer-344 rat where age related death of photoreceptor cell in the peripheral retina has been demonstrated. Peripheral retinas of 3-month-old rats were transplanted with pigmented and/or nonpigmented 6-8 day-old RPE. Morphometric analyses at 3, 6, and 9 months after transplantation indicated a significant delay in the age related cell death in the outer and inner nuclear layers and the two plexiform layers. Up to 9 months after transplantation, the grafted eyes showed significant neuronal rescue in the various layers. RPE transplantation appears to have beneficial effects on the aging eye.

452.11

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452.10

Embryonic rodent retinae transplanted over the midbrain of newborn host rats establish connections with host's olivary pre-tectal nucleus (OPN), an obligatatory nucleus in the neural circuit subserving the pupillary light reflex. Pupilloconstriction can be elicited in the host by direct illumination of the transplant, and transplant-mediated responses are enhanced when host optic input is eliminated. No such change is observed in the pupillary reflex of control rats in response to illumination of the contralateral eye after intracranial sectioning of the ipsilateral optic nerve.

Anatomical studies after labelling the retinal projections of the transplant and host eye (in experimental rats) or both eyes (in normal rats) with different markers show that the transplant and host inputs converge in the host OPN, while inputs from the eyes of normal rats are segregated to different regions of the nucleus. The convergence of transplant and host inputs may be reflected in the interaction of transplant and host inputs to determine host pupil diameter and provides an anatomical basis for the rapid enhancement of transplant-mediated pupilloconstriction when host optic input is eliminated. Segregation of the left and right retinal inputs in normal rats, on the other hand, may serve to limit the functional interaction of inputs from the two eyes.

Supported by NIH grant EY 05283.

452.12
DIFFERENTIAL SURVIVAL OF SENSORY ELEMENTS IN INTRACRANIAL OTOIC TRANSPLANTS: E.J. Hughes and M.C. Silverman. Sensory Neuroscience Lab, Central Institute for the Deaf, St. Louis, MO 63110.

We have previously shown that isolated embryonic rat inner ear can be transplanted to the anterior chamber of the eye of an adult. Initially, E15 inner ears were transplanted in isolation. While the développement o f the transplanted organ is dependent on the presence of a host's cochlear duct, transplanted vestibular structures develop normal, although reduced, in the absence of a host cochlear duct. In the absence of a host's cochlear duct, the inner ear develops only as an inner ear mass. However, when the transplanted inner ear is combined with a portion of an organ of Corti (Hughes and Silverman, 1987, ARO Abstr. 11, 219). When the E15 inner ear is combined with an organ of Corti, the transplanted vestibular development is normal. We have also shown that the postnatal survival of intracranial transplants of embryonic retinae depends on the presence of target cues to guide innervation (Sefton et al., 1983, Dev. Brain Res. 19:531). We have previously shown that isolated embryonic rat inner ear can be transplanted to the anterior chamber of the eye of an adult. Initially, E15 inner ears were transplanted in isolation. While the development of the transplanted organ is dependent on the presence of a host's cochlear duct, transplanted vestibular structures develop normal, although reduced, in the absence of a host cochlear duct. In the absence of a host's cochlear duct, the inner ear develops only as an inner ear mass. However, when the transplanted inner ear is combined with a portion of an organ of Corti (Hughes and Silverman, 1987, ARO Abstr. 11, 219).

In order to investigate the possibility that more mature brain might exert a similar supporting role on cochlear development, we transplanted E15 inner ears to either the cortex or the brainstem of a newborn rat. After survival times ranging from 3 to 5 weeks, we found that more than half the transplants survive and develop sensory structures. Transplants made to the cortex only showed evidence of the crista and macular surfaces of the vestibular division. However, these transplants placed within the brainstem demonstrated organotypic development of the cochlear duct, with an organ of Corti apparent on the basilar membrane.

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543.2 NORADRENALINE RELEASE FROM GRAFTS OF SUPERFICIAL CERVELRAL GANGLIA OR FETAL LOCUS COERULEUS TUMORAL CHROMAFFIN TISSUE INTO HIPPOCAMPAL LESION CAVITIES ON ACQUISTION OF CUE OR PLACE TASKS IN RATS WITH HIPPOCAMPAL LESIONS. T. Woodruff*1,2, R. Johnson1, and J. Curtis1. 1Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109; 2Dept. of Psychology, Western Michigan University, Kalamazoo, MI 49008.

In the present study, we examined the behavioral and biochemical effects of transplanting cholinergic neurons derived from the stratum of fetal rats. Embryonic striatal neurons were transplanted into the hippocampal formation of adult rats with limbic-kortex lesions. Cholinergic neurons were entrained in the lesioned hippocampus and showed distinct regional differences in their distribution. The results of this study suggest that the transplant of cholinergic neurons into the hippocampus may be a viable treatment for hippocampal lesioned patients.

We have derived monoclonal antibodies which give complementary staining patterns in the dentate molecular layer: OM-1 to OM-4 selectively recognize the outer two-thirds (the entorhinal terminal field), and IM-1 the inner one-third (the commissural-associational zone). OM-1 recognizes a 93 kDa glycoprotein antigen, OM-2 to OM-4 a second glycoprotein of 36 kDa, and IM-1 multiple antigens of 125-300 kDa.

After unilateral entorhinal lesions, OM immunostaining was abolished in the ipsilateral entorhinal afferent zone of the dentate gyrus. At the same time, the IM-immunoreactive inner zone acquired OM-staining and expanded by 50 μm in width, while the distal zone shrank by 80 μm. We grafted embryonic entorhinal cortex were placed in the entorhinally deafferented distal zone of adult hosts. 14 out of 26 cases showed restoration of OM-immunoreactivity which was restricted to a band in the middle third of the molecular layer. However, staining for the antigen recognized by antibodies OM-2 to OM-4 was restored throughout the entire outer two-thirds of the dentate molecular layer.

Our results suggest that the membrane-associated glycoprotein antigen detected by the OM monoclonals may be part of an intrinsic signalling system used in normal development and in reinnervation of the adult by embryonic entorhinal transplants to direct and restrict the distribution of entorhinal axons to the appropriate part of the dentate molecular layer, the distal two-thirds. This hypothesis is currently being tested in vitro, using roller tube cultures of hippocampal slices.

453.8 DENDRITIC GROWTH FROM FETAL HIPPOCAMPAL CELLS TRANSPLANTED AFTER TRANSIENT FOREBRAIN ISCHEMIA IN RATS AS DEMONSTRATED BY MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) IMMUNOHISTOCHEMISTRY. Morimizu Koshimizu*, Yochi Katsuyama, Shihzi Miyazaki, Takashi Tsubokawa*. Department of Neurological Surgery, Nihon University School of Medicine, Tokyo 171, Japan.

Cell suspensions prepared from 17-18 gestational hippocampi were transplanted into the area juxt above the hippocampus of male Wistar rats (300-350g) following transient forebrain ischemia, and the dendritic growth patterns of the transplanted cells were examined by microtubule-associated protein 2 (MAP2) immunohistochemistry. The ischemic insult was carried out by hemorrhagic hypotension and bilateral occlusion of the carotid arteries at 7 days before transplantation. Four weeks after transplantation, the animals were perfused transcardially and fixed in 2% paraformaldehyde, and the brains were removed and embedded in paraffin.

Consecutive paraffin sections 5μm thick in the thalamus were stained by the ABC method, employing the anti-serum rabbit anti-μMAP2 polyclonal antibody (Peninsula Laboratories Europe) and anti-calbindin D monoclonal antibody (Sigma). It was found that more transplanted cells survived and extensive dendritic growth occurred when complete death of CA1 pyramidal cells had been induced by the ischemic insult. The dendrites demonstrated growth patterns: the cells located in the dorsal part gave off dendrites towards the dorsal direction, and the dendrites of the cells located in the ventral part oriented themselves towards the ventral direction, much like the parallel arrangements of apical dendrites of normal CA1 pyramidal cells. These findings suggest that dendritic growth from transplanted hippocampal cells is dependent on the presence of host hippocampal cells and is guided by the presence of remaining fine terminals of different fibers.


Pieces of fetal midbrain raphe tissue were transplanted into the third ventricle near the dentate gyrus of adult host rats that had previously been denervated by treatment with 5,6-dihydroxytryptamine. In another experiment, the fetal midbrain raphe tissue was co-grafted with the fetal hippocampus including the dentate gyrus in the anterior chamber of the eye of adult rats. One and three months after transplantation, the extent of axonal outgrowth of the tissue into the hippocampus was studied using serotonin and tyrosine hydroxylase (TH) immunohistochemistry. Serotonin fibers were densely distributed throughout the raphe graft tissue, while restricted to an area near the somata of TH positive neurons. A large number of serotonin fibers were distributed in the hippocampus, especially in the molecular layer of dentate gyrus, while only a few TH fibers were observed in the hippocampus. These observations suggest that the serotonergic and dopaminergic neurons located in transplants of midbrain raphe have different reinnervation patterns in the hippocampal tissue.


To explore aspects of eyeblink conditioning which are most impaired by processes of aging, we compared the performance of young (Y) and older (O; minimum of 24 months old) rabbits in the 400 and 750 msec CS-US interval delay paradigms. For all rabbits a 1 Khz, 85 dB tone was the CS, and a psi, 100 msec corneal airpuff was the US. Twenty-four Y and 18 O rabbits were run for 90 trials/day until they attained a learning criterion of 8/9 CRs and then were overtrained a minimum of one day. Both O and Y rabbits required significantly more trials to reach criterion with a CS-US interval of 750 msec (F = 14.88, p< .01). In the 400 msec paradigm O rabbits required 300 trials to criterion, while they required 713 trials to criterion in the 750 msec paradigm. Y rabbits required 171 and 343 trials to criterion with a 400 and 750 msec paradigm, respectively. These age differences were highly significant (F= 37.67, p< .01). The age by paradigm interaction was significant at the .1 level (F= 3.47), suggesting that O rabbits are more impaired at the longer 750 msec CS-US interval. Longer CS-US intervals impair acquisition in rabbits, and processes of aging exacerbate the difficulty at longer intervals. Supported by a Research Incentive Fund Grant from Temple University.


Male Fischer 344 rats aged 5 and 24 months were subjected to 10 behavioral tests for a total of 22 variables, including spontaneous activities, memory, and attention.

The behavioral performances of the two age groups were significantly different in most tests, indicating a clear factorial role of the aging process. Principal components analysis has evidenced a first factor describing some 40% of the information concerning the 22 variables with a 100% discriminating efficiency between the two groups. This first component may be taken as a comprehensive measure of the animal's "behavioral age". On the contrary, the second analysis performed separately on the two groups revealed a relationship pattern between variables, which was different for the two ages, thereby indicating different meanings for the same parameters and their different behavioral utilisation in the two classes. With the aid of the spin glasses physical model, these results lead to the definition of the difference between causal linking and correlations driven by an external parameter.

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454.3 TREATMENT OF D-AMPHETAMINE REDUCE AGE-RELATED MEMORY DEFICITS IN RATS. G.I. Dunbar, G.A. Smith, I.S. Less, B.J. Snyder, and L.A. Mead. Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI 48858. Since previous studies have indicated that age-related memory deficits may be a result of reduced levels of catecholamines, we have attempted to counteract these behavioral deficits by treating rats 6, 12, or 24 months of age with either 0.5, 1.0, or 2.0 mg/kg d-amphetamine or saline (control). All rats were tested for 8 days on a Morris water maze task. Daily IP injections were given 20 minutes prior to testing. Rats were perfused 24 hours following the final testing day and their brains were processed using cytochrome-oxide (CVO) histochemistry. Both the 12 and 24 month-old control rats took more time and swam further searching for the platform than did the 6 month old controls. These deficits were reduced by treatments of 1 mg/kg d-amphetamine in the 24 month old rats, while the 2 mg/kg dose caused further impairments in these aged rats. Age-related deficits in CVO activity were observed in the dorsalmedial thalamic nucleus, nucleus basalis, and caudate nucleus of the 24 month old rats and in the dorsalmedial thalamus of the 12 month old rats, irrespective of treatment. However, these histochemical deficits did not correlate significantly with either of the behavioral measures used, suggesting that amphetamine is effecting behavior in other ways.

454.4 PRINCIPAL COMPONENT ANALYSIS HELPS TO CLARIFY THE STRUCTURE OF A DATA SET: A CASE STUDY ON FOUR BEHAVIORAL PARAMETERS. S. Agresti, A. Giustini, F. Ficaras, Sigma Tau Institute for Research on Senescence, Pomezia, Rome, Italy. sprague Dawley rats (N = 80) of different ages, fed standard or hypocaloric diet, were subjected to a radial water maze task the effect of age and diet on their performances. Four performance indices were singled out from the test: 1) Spatial reference memory 2) Spatial working memory, 3) Non spatial reference memory 4) Non spatial working memory. These parameters, measured over 10 subsequent sessions, were globally analyzed with the aid of the principal component analysis (PCA), which highlighted a first factor explaining 65% of total variability. This factor was highly correlated with time course as measured by the successive sessions (R = 0.9). Such a relation individuated the factor as "learning"; all the parameters had a significant factor loading on this component. When PCA was performed separately at each session, then eliminating the "learning" effect, the 4 kinds of memory resulted to be completely uncorrelated, thence pointing out to the role of "order parameter" exerted by learning with respect to the measured parameters.

Aging was demonstrated to decouple learning and working memory performances and this decoupling was antagonized by an hypocaloric diet.

454.5 HIPPOCAMPAL MOSSY FIBER ZINC AND SPATIAL MEMORY IN AGED RATS. D. Guidolin*, P. Pizzato*, A. Zanotti*, E. Meccoli*, E. Fabbrini*, M.G. Nusti. Fidia Research Laboratories, 35031 Abano Terme (PD), and 2Center of Immunology, Dept. of Gerontologic Research, IRBCA, 60121 Ancona, Italy. Zinc is known to play a key role in synaptic function of the mossy fiber system, a pathway deeply involved in hippocampal processes related to spatial learning and memory. We evaluated the amount of histochemically detectable zinc in the mossy fibers from young adult (6 month old) and aged (25 mo) rats tested for spatial performance. Computer-assisted microdensitometry and x-ray microanalysis showed a significant difference (40%) on the non-Timer's staining between memory-impaired and non-impaired aged rats, thereby suggesting a correlation between age-associated memory deficits and localized changes in hippocampal zinc levels.

Furthermore chronic oral administration of phosphatidylserine restores plasma zinc levels in aged rats, we are currently evaluating the effects of this phospholipid on hippocampal zinc levels.

454.6 A NEW TEST DESIGNED TO ASSESS MOTOR IMPAIRMENT IN AGED RATS. F. García-Hermández and R. Duchater-Quil. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-364, México, D. F. Neurodegenerative disorders which involve motor impairment is characteristic of old age. Although there are a few tests which attempt to assess motor incapacities, many have utilized scales which have either a great deal of subjective evaluations or are subject to learning-performance complexities. This study describes a method which is designed to measure motor impairment of aging rats which is subject to dopaminergic influences and has negligible practice effects. The test is designed so that rats have to traverse 2 meter beams of 15 mm inclination whose width is 3.5, 2.0, and 2.4 mm are utilized on each test session using a table of random numbers. The time ceiling allowed for traversing the 2 beams was established at 120 sec. The test was given to 3 month old rats and aged rats (5-20) with a mean age of 26.5 ± 3.8 months ranging from 23 to 34 months. All young rats traversed the beams, independently of beam width, while virtually none of the old rats traversed the 3 and 6 mm beams. However, as the beam width increased more and more aged rats ascended the beam. Nevertheless, there were always a few old rats who were unable to cross even on widest beam. When young rats were fitted with a lead belt which increased their body weight by approximately 40%, they still traversed all beam widths. On the other hand, Haloperidol (1.0 and 2.0 mg/kg) severely impaired the performance of young rats. Conversely administration of amphetamine (1.0 mg/kg) or L-dopa (50 mg/kg) to old rats substantially improved their performance. The results suggest that beam taxis appears to be an effective and efficient test for screening motor dysfunction in aged rats and can be amenable to studies on changes of motor capabilities as a result of drugs and/or brain damage.

This work was partially supported by FINESSIN.

454.7 AGE DEPENDENT ALTERATION OF NERVE TERMINAL ACTIVITY AT THE NEUROMUSCULAR JUNCTION (NMJ) OF RAT: A MICROELECTRODE STUDY. M.D. Sokoll, B.J. Bhattacharya* and K. Tsien*. Department of Anesthesiology, University of Iowa College of Medicine, Iowa City, Iowa 52242 Rats have been used by several workers to study synaptic aging. Using the two microelectrode voltage-clamp technique, we studied age induced alterations of ACh release in phrenic nerve-diaphragm preparation of young (3 month old) and old (30 month old) Sprague-Dawley rats. Results of this study indicate that spontaneous release of ACh measured by recording miniature end-plate current (MEPC frequency) was significantly higher in old rats. Quantum content calculated by the direct method (recording MEPCs immediately before the in trains of MEPCs) indicated a larger quantum content in old muscle compared to the young. The EPC quantum content was also studied by recording evoked release (EPC evoked by single stimuli (0.4 Hz from same cell)) indicated a larger quantum content in old muscle compared to the young. The EPC quantum content was also studied by recording evoked release (EPC evoked by single stimuli (0.4 Hz from same cell)) and evoked release (EPC evoked by single stimuli (0.4 Hz from same cell)) and evoked release (EPC evoked by single stimuli (0.4 Hz from same cell)). Since previous studies have indicated that age-related memory deficits may be a result of reduced levels of catecholamines, we have attempted to counteract these behavioral deficits by treating rats 6, 12, or 24 months of age with either 0.5, 1.0, or 2.0 mg/kg d-amphetamine or saline (control). All rats were tested for 8 days on a Morris water maze task. Daily IP injections were given 20 minutes prior to testing. Rats were perfused 24 hours following the final testing day and their brains were processed using cytochrome-oxide (CVO) histochemistry. Both the 12 and 24 month-old control rats took more time and swam further searching for the platform than did the 6 month old controls. These deficits were reduced by treatments of 1 mg/kg d-amphetamine in the 24 month old rats, while the 2 mg/kg dose caused further impairments in these aged rats. Age-related deficits in CVO activity were observed in the dorsalmedial thalamic nucleus, nucleus basalis, and caudate nucleus of the 24 month old rats and in the dorsalmedial thalamus of the 12 month old rats, irrespective of treatment. However, these histochemical deficits did not correlate significantly with either of the behavioral measures used, suggesting that amphetamine is effecting behavior in other ways.
454.9

FURTHER ANALYSIS OF SENSORDIATOR MUSCULAR DYSFUNCTIONS IN AGED RATS: ADHESIVE PAPER TEST. T. Schuwerk* and M. de Jonge. Tropenmedizin, Dept. for Neurology, Univ. of Gerontopharmacology, Berliner Str. 156, 5000 Köln 60, FRG.

Until now little research has been directed to the study of age-related sensorimotor dysfunctions compared with the effects of age-dependent cognitive deficits. Our previous studies showed an impairment of motor coordination of old rats subjected to balance rod and pole running tests. An analysis of the footprints of aged rats showed a disturbance of the coordination of the movements of the hindlegs during walking. In two experiments we detected differences between habits and old rats in stimulus-directed movements of the forepaws. In the first experiment, small pieces of adhesive paper were placed on various parts of the rats’ snout or forepaws. The time needed to remove the sticky paper was measured. 25-month-old rats needed 2-4 times longer than 3-month-old rats to remove the stick. In the second experiment young and old rats were repeatedly subjected to the adhesive paper test. Latencies to remove the sticky paper decreased significantly in 3-month-old rats. In contrast, 25-month-old rats showed no improvement of their sensorimotor skills.

454.11


The present study examined the effects of long-term muscle usage on axonal sprouting (intranodal, preterminal, and intraterminal) as well as terminal branch number and endplate area following L3, L4, or L5 partial denervation in rats. We found that in partially denervated (PD) tibialis anterior muscles, there was a progressive increase in all types of axonal sprouting up to 9 months post-surgery as compared to sham-operated controls. The number of terminal branches per endplate in the PD muscles remained constant and less than controls from 1 to 6 months post-surgery, and then increased significantly, doubled from previous values and was about 1.5 times greater than in controls. The endplate area in PD muscles at 3 to 12 months post-surgery was greater than in controls. At 12 months, axonal sprouting and number of terminal branches were decreased compared to sham controls; denervated endplates and endplates exhibiting degenerative changes were also seen in PD muscles. It would appear that there is an earlier onset of aging-like changes in the PD muscles possibly induced by enhanced stress on the remaining motoneurons which are hyperfunctioning due to a greatly enlarged peripheral field of muscle fibers. Supported by NIH grant NS35624.

454.13

SIBERIAN HAMSTERS AGE AT DIFFERENT RATES IN LONG AND SHORT PHOTOPERIODS. C.M. Finley and I. Zucker*, Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

In small mammals dry eye lens weight is a reliable marker of chronological age. Lens weights continue to increase throughout the life span even when body weight remains stable. In Siberian hamsters maintained in either long (LD 16:8) or short (LD 8:16) photoperiods, dry eye lens weight doubled from previous values and was about 1.5 times greater than in long-day animals (29.29 ± 10.26 µg) (2) and 12 hours (35.56 ± 14.91 µm³) were not significantly greater than the mean area under the initial recording taken at 30 minutes post hemisection (29.29 ± 9.67 µm²). The first significant p (0.01) increase of mean integrated area, occurred 24 hours after hemisection (36.29 ± 26.12 µm²). Activity measured at 7 days (86.66 ± 23.56 µm²) and 30 days (99.48 ± 27.65 µm²) was not significantly different from the enhanced activity measured at 24 hours. From these data, we conclude that crossed phrenic nerve activity increases significantly in older rats after spinal cord injury, but the augmentation of the reflex occurs more slowly than it does in young adult rats.

454.10


A recent quantitative electrophysiological study from our lab has shown that there is a significant augmentation of crossed phrenic nerve activity that can be induced as early as 2 hours after spinal hemisection in 3-4 month old young rats (Exp. Neurol. 111:224-230, 1991). The present study utilized the same techniques on older rats (9-10 months old) to determine if the time course for the augmentation of the reflex between the two age groups was similar. Mean integrated areas under phrenic nerve compound action potentials during the crossed phrenic reflex at 4 hours (26.60 ± 6.62 µm²) and 12 hours (35.56 ± 14.91 µm³) were not significantly greater than the mean area under the initial recording taken at 30 minutes post hemisection (29.29 ± 9.67 µm²). The first significant p (0.01) increase of mean integrated area, occurred 24 hours after hemisection (36.29 ± 26.12 µm²). Activity measured at 7 days (86.66 ± 23.56 µm²) and 30 days (99.48 ± 27.65 µm²) was not significantly different from the enhanced activity measured at 24 hours. From these data, we conclude that crossed phrenic nerve activity increases significantly in older rats after spinal cord injury, but the augmentation of the reflex occurs more slowly than it does in young adult rats.

454.12

THE EFFECTS OF DIFFERENT LIGHT CYCLES ON LIFESPAN IN CARDIOMYOPATHIC HAMSTERS. S.D. Drastal*, A. Berenfeld, J. H. Nelleson, W. N. Tapp, and J. E. Ottenweller*. Dept. of Neurosci., N.J. Medical School and VA Medical Center, East Orange, NJ 07019.

Our earlier work showed that hamsters with heart disease lived significantly longer in an environment devoid of time cues than in light/dark (LD) 12:12. To evaluate these results, male cardiomyopathic hamsters (CMHs) were housed in four lighting conditions: LD 12:12, LD 6:18, LD 6:30, and LL (constant light). In addition, CMHs were housed in LD 12:12, five/five, but in larger cages. The LD 12:12 and five/five groups were chosen to replicates our original finding while the other groups were chosen to evaluate the effects of a short photoperiod (LD 6:18) and an entrained non-24 hour LD schedule (LD 6:30) on lifespan. Survival curves were generated and tested for statistical significance compared to the LD 12:12 two/five group. Compared to that control group, LL extended life over the entire survival curve, LD 6:18 did so over the first half of the survival curve, and LD 12:12 five/five group did so over the second half of the curve. These data confirm our earlier work on the life-extending effects of constant light and suggest that other environmental factors related to either cage size or animal density also are important. Supported by VA Medical Research.

454.14

NOICEPTION INCREASES AND THE EFFECTIVENESS OF ANALGESICS DECREASE IN AGING. E. E. Quilton. Lab of Psychobiol., Univ. Louisville, Louisville, Kentucky 40292.

The present study was undertaken to further characterize the age-nociception interaction in the mouse. Two age groups of C57BL/6J mice were used; old (27-30 mo) and adult (8-12 mo). In exp. 1 mice from both groups were inj with saline, morphine (15 mg/kg) or CCK (400 μg/kg) and tested on a hot plate (55°C) 5 times from 10 min to 120 min after inj. The old-sal group was more responsive than 30 min after inj but did not differ from the adult-sal group thereafter. CCK was analgesic in both groups through 45 min, but the old and adult groups did not differ. Morphine was more analgesic in the adults throughout, but CCK was less analgesic elsewhere. In exp. 2 the formalin test for chronic pain was used and paw-lick was monitored for 60 min after inj of the formalin. Mor. (2 mg/kg), CCK (400 μg/kg), or sal was inj in 5 min before the formalin. The old-sal group had a higher rate of paw-licking than the adult-sal group, but CCK was more effective than the old-sal group. Mor. was less effective in the old. CCK produced a short term analgesia followed by a rebound pain, and had a stronger effect than mor. in the old group over the duration of the test interval. In exp. 3 & 4 a novel analgesic and tolerance to mor. was less in the old mice.
545.1 TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-1β INCREASE THE PHOSPHORYLATION OF TWO 30,000 M, (HEAT SHOCK) PROTEINS IN ASTROCYTES. P. E. Millhorn* and D. Y. Combs. Dept. Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

Cytokines affect various aspects of astrocyte function but little is known about the action of these agents on protein phosphorylation. Cultures of rat astrocytes were labeled with [32P]orthophosphate for 2 h and then treated with 1 ng/ml interleukin-1β (IL-1β), 100 U/ml interferon-γ (IF-γ), or 100 ng/ml tumor necrosis factor-α (TNF-α) for 10 min. Treatment with IL-1β and TNF-α increased 32P-incorporation into two 30,000 M, proteins with pl values of 5.7 and 5.5, however, no effect was observed with IF-γ. Based on the apparent molecular weight, pl values, and phosphorylation response to other agents, these 30,000 M, proteins are likely the low molecular weight heat shock proteins (HSP). Treatment with protein kinase C (PK-C) activators also increased 32P-incorporation into these proteins, however, the effects of IL-1β and TNF-α are observed in PK-C-depleted astrocytes suggesting that their action is not mediated by PK-C. The 30,000 M, proteins can also be phosphorylated in response to agents which mimic or increase cAMP along with a number of other proteins including glial fibrillary acidic protein (GFAP) and vimentin. However, GFAP and vimentin are not phosphorylated in response to TNF-α and IL-1β suggesting that their action is not mediated by cAMP-dependent protein kinase (PK-A). These studies suggest that protein phosphorylation, mediated by a signal transduction pathway independent of PK-C and PK-A, may play an important role in the action of TNF-α and IL-1β in astrocytes.

545.2 IMMUNOCALCIFICATION OF CALPN II IN RAT PERIPHERAL NERVE. M. Kato and D. J. Fink. Dept. Neurology, University of Michigan, Ann Arbor, MI 48109.

Calpain II is a thiol protease which is activated by millimolar Ca2+. The protein is a dimer composed of 80 K catalytic subunit and a 30 K regulatory subunit. We have used a rabbit polyclonal antibody raised against rat platelet calpain II (a gift of J. Elce) which recognizes only the 80 K subunit in Western blot of homogenate of rat peripheral nerve.

Light microscopic immunocytochemistry of 1 micrometer cryo-sections of adult rat sciatic nerve using biotin streptavidin peroxidase reaction revealed prominent staining of the Schwann cell cytoplasm which extended to the pia-arachnoid of the Schwann cell. Immunoreactivity was seen in the myelin, or in the axon using this method.

Electron microscopic immunocytochemistry of cryo-ultrathin sections using secondary antibodies bound to colloidal gold confirmed diffuse staining of Schwann cell cytoplasm. There was prominent staining of the plasma membrane and along the external surface of the Schwann cell.

These results suggest that calpain II in nerve may be exposed to the millimolar Ca2+ concentration required for activation in the extracellular space, and may therefore play a role either at the Schwann cell membrane or in modulation of the extracellular matrix.


The calcium-activated protease calpains are involved in the pathological process in spinal cord injury. By immunocytochemical and immunoelectron-microscopic technique, we observed the morphological distribution of micalpain in CNS. micalpain immunoreactivity (IR) is widely distributed in CNS.

In spinal cord, micalpain-containing structures include neurons, fibers, glia. The IR neurons with weak staining are in laminae VII, IX, X and X but the cell membrane and nuclear membrane of motoneurons are distinct. Sparse numbers of IR fibers appear in white and dorsal root. IR in particular fibrous astrocytes with the strongest staining were seen in both gray and white matter and were most dense at the surface of the spinal cord just beneath the pia mater.

In the brain, a few weak-staining IR neurons and sparse fibers are in hypoglossal, vestibular nuclei, nuclei of trigeminal spinal tract and solitary tract, reticular formation, substantia nigra, hypothalamus, purkinje cells, dentate nucleus and layer V, VI of neocortex. Glia, ependyma and endothelia stained most prominently. Supported by NS-11046 and NS Soe 2130.

We are interested in the extrinsic signals that may lead to specific neuronal shape along the frequency axis of a central auditory nucleus, the lateral superior olive (LSO). Antibodies directed against glial fibrillary acidic protein (GFAP) and glial fibrillary acidic protein (GFAP) were employed on sections of paraffin embedded tissue from adult gerrils (Meriones unguiculatus), and visualized with an avidin-biotin-peroxidase amplification system (Vector). In addition, the density of astrocytes was determined from toluidine blue stained semi-thin sections through the LSO.

GFAP immunoreactivity was restricted to the cytoplasm of small non-neuronal cells. The number of GFAP-positive cells appeared greater in the low frequency region of the LSO. GFAP immunoreactivity was more prominent in astrocytic processes than their cell bodies, and some of these processes terminated on the blood vessel endothelium within the LSO. The GFAP immunoreactivity was much greater in the low frequency region of the LSO: Preliminary counts of GFAP-positive cells showed a threefold difference between the low frequency and high frequency regions. Preliminary counts of all astrocytes in toluidine blue stained material also showed a heterogenous distribution. The present results suggest that giall cell distribution along the frequency axis of the LSO is correlated with a known difference in dendritic form. (Supported by NIH NS26676-01A1; DHS is a Sloan Foundation Fellow)

455.7 RELEASE OF CALCIUM FROM INTRACELLULAR STORES OF ISOLATED RETINAL GLIAL (MULLER) CELLS. S. A. Keirstead and R. F. Miller. Dept. of Physiology and Neuroscience Graduate Program, University of Minnesota, Minneapolis, MN 55455.

Previous studies in our laboratory have shown that dissociated Muller cells from the tiger salamander respond to an elevation in extracellular potassium ([K+]c) with an increase in intracellular calcium ([Ca++]i), even in the absence of Ca2+ in the extracellular medium. This suggests that the K+-evoked depolarization causes a release of Ca++ from intracellular stores. In the present experiments, we have used Fura-2 imaging techniques to study the calcium release mechanism in single dissociated Muller cells from the tiger salamander retina.

Muller cell responded to bath application of caffeine (10 mM) in Ca-free Ringer (2 mM EGTA) with an increase in [Ca++]i, Ryanodine (10-50 gM; Caffeine) also evoked an increase in intracellular Ca concentration, and reduced or blocked Muller cell response to caffeine (10 mM). The changes in [Ca++]i evoked by caffeine and caffeine were more prominent in the apical region and soma of the Muller cell and less evident or delayed in the specialized endfoot region. This same spatial pattern was often observed in response to an elevation in [K+]c, in the absence of [Ca++]i. These findings suggest the presence of a ryanodine-sensitive mechanism in Muller cells which may be involved in the K+-evoked increase in [Ca++]i. (Supported by NEI grant EY03014)


Glionexin (GX), a high molecular weight extracellular matrix (ECM) glycoprotein recognized by the 52k antibody (Meyer et al., J. Neurosci. 7:512 (87); Dev. Biol.130:374 ('88)), is associated with discrete types of glia in the CNS of embryonic and adult cricket (Acheta domestica). We now show that GX is also expressed selectively in the peripheral nervous system. GX is found in: (a) mechanoreceptor hairs (cercal filiform and clavate sensillae of the chondrid organs (submucal, tympanic, femoral, Johnston’s) in legs and antennae. GX is excluded from fine trichoid mechanoreceptor organs on cerci, antennae, legs, and palps, as well as other glia and photoreceptor organs. Therefore, GX expression in the periphery is sensorily modality selective.

GX is transiently expressed on basal lamina of embryonic ectoderm, and is expressed in the mechanoreceptor organs in developing limbs and cerci; mechanoreceptor labeling persists throughout development. Peripheral GX immunoreactivity appears to be more widespread than the ECM labeling in sensory organs. Highest levels of GX expression are usually associated with either ECM regions surrounding sensory neuron somata or with sheath cells which wrap sensory types of mechanoreceptor organs show differential patterns of GX labeling. Thus, peripheral GX expression may be both receptor class- and non-neural cell type-selective. Supported by NS-07778.

455.10 SECRECTION OF PEPTIDES BY CHOROID PLEXUS AND EPENDYMAL CELLS IN CULTURE. R. H. Angeletti, P. Gee* and C. H. Rhodes, Albert Einstein College of Medicine, Bronx NY 10461 & Dartmouth Medical School, Hanover NH 03756.

Immunohistochemical studies with sequence-specific antibodies to the peptide/glycine alpha-amidation enzyme (PAM) and carboxyamidase (CPE) demonstrated that this enzyme is found in glial cells: Schwann cells, astrocytes, ependyma, choroid plexus, subependymal and subpial glia [J. Histochim. Cytochem. 38:1301 (90)]. These data are consistent with recent reports that the endocannabinoid, arachidonic acid (AA), another neuropeptide processing enzyme, is also present in glia [Birch et al., Mol Brain Res. 7:3 (90); MacCumber et al, J. Neurosci. 10:2850 (90)]. In order to study their secretion, we have set up primary cultures of rat ependyma and choroid plexus, as well as cultures of a choroidal plexus cell line. Pulse-chase experiments with [3H]methionine, [3H]-glutamic acid, [3H]-leucine, and other labeled amino acids show that a discrete set of polypeptides is secreted, ranging in molecular weight from >200,000 daltons to less than 10,000 daltons. These peptides are being isolated, so that their identity can be determined. Neuropeptide processing enzymes are also being measured by activity, cDNA and immunoaffinity probes. Further studies have demonstrated the presence of PAM immunoreactivity in human ependyomas. (Supported by NIH grant NS-22697)
455.11

Peptidylglycine amphoteric monooxygenase (PAM), an enzyme involved in neuropeptide biosynthesis, is present in normal glia and gliomas. C. H. To, R. R. Schachter, R. A. Williams, and J. A. Wolfe. Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, MD.

† Determining the signals involved in regulating the proliferation of astrocytes is important to an understanding of normal brain development as well as pathological processes such as reactive gliosis. Our laboratory has previously reported that recombinant human glial cell line-derived neurotrophic factor (rhGDNF) is mitogenic (7-fold) for adult human astrocytes in vitro. PAM has been demonstrated not only in neurons, but also in Schwann cells, normal ependyma and some glia (J Histochem. Cytochem., 38:1301-1311 (1990)). The present study shows the production of PAM is also secreted by astrocytes. This was demonstrated by experiments which showed the presence of PAM was mitogenic (3-fold) for adult human astrocytes in vitro. In primary astrocyte cultures derived from aged murine astrocyte cultures by treatment with dexamethasone (dex), Masri #6 expressed assemblies in the presence or absence of dex. Thus, clonal astrocytic cell lines may resemble astrocytes as visualized with phase contrast optics, but have significant differences in the expression of several epitopes and in membrane structure regulation. Assessing the utility of such cell lines for isolation of cell-specific cDNAs requires careful characterization of specific cell lines and culture conditions.

455.12

ORGANIZATION AND STRUCTURE OF ASTROCYTES IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. Lucas D. Pozzo Miller* and Dennis M.D. Landis, Depts. of Neurology and Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106-4901.

In organotypic cultures, the initial cellular relationships at the time of plating are essen tially those that existed in vivo. We have prepared organotypic cultures of hippocampal slices obtained from 6-7 days old rats and maintained for 6-8 weeks in vitro. Astrocytes in such slices phagocyte large amounts of cellular debris, and appear to form a glial lamina. Among the neuronal elements, however, the patterns of astrocytic investments resemble those present in developing hippocampus. In cultures prepared by aldehyde fixation and freeze fracture techniques, astrocytic processes in the superficial 15-20μm of the slice are characterized by very large gap junctions, and numerous "assemblies". These arrays of orthogonally-polarized intramembrane particles are normally found in astrocytic processes investing blood vessels or forming the glial lamina in situ. Astrocytes in secondary cultures also express assemblies, but with lower densities than those observed in tissue or in organotypic cultures. Cytoplasmic organization in astrocytes appears similar in cultures prepared by rapid freezing and freeze substitution fixation. We find that selective neuronal death occurs in particular regions and at particular times in the cultured slices. These cultures provide an opportunity to examine the response of astrocytes to both the initial damage of making the slice, and to subsequent neuronal death, in the relative or complete absence of blood-borne macrophages.

455.14

IN SITU AND IN VITRO EXPRESSION OF TENASCIN BY SUPRAOPTIC NUCLEUS ASTROCYTES. A. K. Salim and P.S. Klinkhammer. Departments of Anatomy, West Virginia University, Morgantown, WV 26506.

The dendritic arbors of hypothalamic supraoptic nucle us (SON) neurons of adult rats undergo activity-related remodelling to form bundles. Tenascin is an extracellular matrix glycoprotein that is expressed by astrocytes, primarily during development. In developing SON, it is believed to play a role in histological patterning by serving as a "boundary molecule" to outgrowing neurites. The morphological plasticity of the adult SON suggested the hypothesis that astrocytes in the adult SON express this morphogenetic molecule. Twelve adult men were anesthetized, perfused with fixatives and histologically processed. Double-label immunocytochemistry with primary antibodies against tenascin and glial fibrillary acidic protein (GFAP) was used to assay 5-10μm thin sections through the SON. We observed a consistent, generally overlapping, staining for tenascin and GFAP of those SON astrocytes with cell bodies in the subjacent glial lamina and processes extending into the nucleus. Staining was usually confined to fibers of the glial laminae and dendritic zone, but in four animals was seen to encircle neuronal cell bodies. Primary cultures prepared from the SON produced both flat and stellar GFAP+ astroglia. When immunostained for tenascin without permeabilization, a subpopulation of these cells clearly expressed a surface patchwork of the molecule. With permeabilization however, nearly all GFAP+ cells displayed tenascin+ perinuclear "granules", the distribution of which closely paralleled that of the glial filaments. These results indicate that some astrocytes whose processes distribute in the adult SON synthesize and express tenascin. The in vivo results suggest that synthesis and surface expression of this glycoprotein may be differentially regulated. Whether and how the presence of tenascin in the SON is related to structural remodelling, and the possibility of tenascin expression by other SON cell types, remains to be determined.

455.15

AGE AND SPECIES DETERMINANTS OF PROLIFERATION OF ASTROCYTES TO RECOMBINANT HUMAN GDNF (RHDNF) INTERFERON. T. Tejada-­ Bergroth, F.P. Yong and V.H. Yong, Montreal Neurological Institute, Montreal, Quebec, Canada KHA 2B4.

Determining the signals involved in regulating the proliferation of astrocytes is important to an understanding of normal brain development as well as pathological processes such as reactive gliosis. Our laboratory has previously reported that recombinant human glial cell line-derived neurotrophic factor (rhGDNF) is mitogenic (7-fold) for adult human astrocytes in vitro as assessed by GFAP/BrdU double immunofluorescence. Recombinant mouse glial cell line-derived interferon (mIFN) inhibits the proliferation of cultured neonatal mouse astrocytes as measured by autoradiography (50% reduction at 100 μl/ml). The results from the immunofluorescence method of BrdU incorporation correspond well with measurements of [3H]-thymidine. In order to determine whether the differential response to rhIFN and mIFN is due to species- or specific-dependent, human fetal astrocytes were examined for their response to rhIFN. rhIFN (10-1000 μml/ml) induced a 2-fold increase in the proliferation of human fetal astrocyes in vitro as evaluated by both BrdU and [3H]-thymidine incorporation. We are currently testing the mitogenic capacity of mIFN on cultured adult murine astrocytes. Preliminary data suggest that differential effects of gamma-IFN on human and murine astrocytes reflect species-specific differences.
455.17 INFLUENCE OF GRAFTED PURKINJE CELLS ON BERGMANN GIAL
ENZYME EXPRESSION: M. Fisher and P. Trimmer. Dep'ts of Anatomy &
Cell Biology and Neuroscience, University of Virginia, University of Virginia,
Charlottesville, VA 22908.
Postnatal loss of Purkinje cells (Pcs), Bergmann glia (Bg) in the Lurcher (Lc) mouse cerebellum show a striking metabolic response that includes decreased expression of the enzyme glycerol-3-phosphate dehydrogenase (GPDH). Analysis of Lc chimeric mice showed that Lc Bg were competent to maintain normal GPDH levels as long as they were situated within the domain of at least one surviving wild-type Pc (Fisher, M., 1995, J. Neurogenet. 6:183.). Grafts of wild-type fetal Pcs into Lc mutant cerebellum have been used to determine whether already and down-regulated Bg can be reinduced to express a high level of GPDH. Grafts were made into hosts 3-6 weeks old and analyzed 6-8 weeks after surgery. Only grafts into very young hosts result in significant observable glial GPDH immunoreactivity. The host age restriction and the distribution pattern of GPDH-positive Bg suggest that grafted Pcs can sustain GPDH expression in Bg that have not completely shut down, but they cannot reinduce expression in those cells that have already shut down. EM analysis shows a significant reduction in the extent to which Bg enwrap dendrites of grafted Pcs relative to that seen in normal cerebellum. The results are consistent with the idea that Bg require continuous extensive interaction with Pcs to maintain normal adult levels of GPDH. Supported by NS25350 from NINDS.

455.19 QUANTIFICATION OF THE CELLULAR GROWTH BALANCE OF GLIOMA
CELLS USING MULTIPARAMETER FLOW CYTOMETRY. Herbert H.
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Northwestern University, Chicago, IL 60611.
Proliferating cells in the process of balanced cellular growth double their protein content before entering mitosis. Alterations in cellular growth balance produce changes in the ratio of total cellular protein (TCP):DNA, and the expression of specific proliferation-associated proteins. In this study, a new three-color, dual-laser flow cytometric technique was used to measure cellular DNA, TCP and c-myc protein content using cell staining with the fluorescent dyes DAPI, SR101 and FITC. Modulations in the cellular growth balance of A-172, U118MG and U138MG (human glioma) cells were produced using growth-inhibitory conditions including serum deprivation, stationary phase growth and treatment with DMSO and sodium butyrate (SB). Flow cytometric data were confirmed using immunohistochemical and the Lowry method.

Growth-inhibited cells exhibited changes in DNA, TCP and c-myc protein content which could be quantified using the new technique. SB-treated and stationary phase A-172 cells exhibited the most pronounced rearrangement in cellular growth balance. In these cells, the ratio of protein content in G2/M to G0/G1 cells increased from 1.43 to 1.70 (+0.05), while c-myc protein/TCP dropped from 1.00 to 0.31 (+0.02). The new dual-laser flow cytometric method should be useful for understanding other complex cell processes.

455.18 ENZYME EXPRESSION IN BERGMANN GLIA OF ADULT MICE
REMAINS SENSITIVE TO PURKINJE CELL PRESENCE. G. Ruthel and
M. Fisher. Department of Anatomy and Cell Biology, University of Virginia,
Charlottesville, VA 22908.
Bergmann glia (Bg) in certain mutant mice respond to the early loss of Purkinje cells (Pcs) with marked metabolic response involving a reduction in glyceral-3-phosphate dehydrogenase (GPDH) levels. Studies with mutants suggested that normal glial enzyme expression requires the presence of Pcs at least through the first postnatal month (Fisher, J., Neurogenet. 6:183, 1990). To determine whether glial GPDH expression requires the presence of Pcs in adults, intracerebellar injection of kainic acid (1 uL, 0.5 mg/mL) was used to produce areas of selective Pc loss in mice at 7, 10, and 13 weeks of age. By 3 to 4 weeks post injection, there was a noticeable decrease in GPDH immunoreactivity in Bg in areas devoid of Pcs. By 8 weeks post injection, GPDH immunoreactivity was almost entirely absent from glial cell bodies and reduced in the glial processes. The presence and normal appearance of radial Bergmann fibers in the affected areas was confirmed in sections stained with antibody to glial fibrillary acidic protein. In some cases, isolated Bg of high GPDH immunoreactivity were observed within an affected area. Visualization of Pcs in adjacent sections with antibody to calbindin showed such Bg to be within the dendritic field of a similarly isolated Purkinje cell. These results suggest that normal glial GPDH expression in adults depends on the continued presence of Purkinje cells.
456.3 TEMPERATURE EFFECTS ON GROWTH OF GRADIENT PURIFIED MURINE CNS CELLS IN VITRO: RAPIDLY PROLIFERATING GFAP EXPRESSION IN ASTROCYTES AT 34°C DIFFER FROM SLOWLY PROLIFERATING CELLS WITH LOWER GFAP CONTENT

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Temperature sensitive retroviruses provide a means to study the pathogenesis of neuronal and neurodegenerative diseases induced by mutant neuronal retroviruses [Neural 4(Suppl 1):143, 1991]. In order to study the specific vulnerability of CNS cells to these retroviruses, in vitro, purified CNS cells were obtained from 5-10 day old FVB mice. Cerebral cortex (CTX) subcortex (CSTX) and cerebellum (CBL) were dissected, enzyme dissociated and separated by density gradient centrifugation into microvessels and other CNS tissue elements [Ann Neural 26:291,1990]. The Dextran separation was assessed by detergent removal through 30% Percoll into three bands at the following densities: 1) 1.018, 2) 1.033 and 3) 1.098 g/ml. Because rapidly proliferating cells may permit more virus replication, proliferation rates of murine CNS cells from CTX, CSTX and CBL were measured at 34, 37 and 39°C. Cells from band 1 grew poorly at all temperatures. Cells in band 3 from all three regions grew significantly (p < 0.03) better at 34°C [4.7 ± 0.6 (standard deviation) x 10^5 cells/cm^2] at 15 days in vitro (DIV) compared to 39°C [2.6 ± 0.9]. Cells in band 3 from all CNS regions did not grow differently at 34°C [2.0 ± 1.4] from 39°C [3.8 ± 1.1]. CNS cells from different regions were characterized in vitro by immunocytochemistry and immunoblotting with specific antibodies for GFAP, a microvessel protein which is fluorescently activated cell sorting with lectins and acetylated low density lipoprotein binding. Rapidly proliferating astrocytes in band 2 had a higher GFAP content than cells from band 3.

456.4 DISTRIBUTION AND MORPHOLOGY OF HIPPOCAMPAL MICROGLIA FOLLOWING CHRONIC EXPOSURE TO ETHANOL. A.L. KAELBLE, S.D. HARVEY, J.E. McBEAN, and J.G. MILLER.* Dept of Neuroscience, Univ. of Florida, Gainesville, FL 32610

Microglial cells are potential immunooptor cells which respond to neuronal injury and death by morphologic and phenotypic transformations. Chronic ethanol treatment (CET) has been shown to produce a loss of neurons in the hippocampus. This study examined the effects of CET on microglial distribution and morphology in the rodent hippocampus. Animals in the ethanol (E) and control (S) groups were fed the appropriate diet (20% plus 28% sucrose for 28 weeks followed by an 8 week period of abstinence. Microglia were visualized in vibrastome sections from the dorsal, central and ventral hippocampi using lectin histochemistry with the Bi6-isolation from Griffonia simplicifolia. Resting microglia in E group animals possessed extensive process staining and were scattered throughout all subfields and laminae, being most prominent in the dentate hilus and entorhinal cortex. Microglia were differentially distributed in various subfields and regions within the dorsal-ventral extent of the hippocampus. On the other hand, microglia from the hippocampi of E-group animals appeared reduced in number with a greater reduction in the dorsal as compared to ventral hippocampus, particularly in area CA1. In addition, animals possessed fewer and more truncated processes than observed in S-group animals. These results suggest that abnormal microglial cell function may be related to the reduction in reactive synaptogenesis following chronic exposure to ethanol. Supported by the Veterans Administration and NIAAA AA00200.

456.5 ATTENUATION OF NEURITE OUTGROWTH PROMOTION AND NCAM AND HNK-1 EXPRESSION DURING IN VITRO MATURATION OF CN STROCYTES. P. Z. Fromk* and J. Jacobberger.
Case Western Reserve University, Dept. of Neurology, Cleveland, OH 44106.

The attenuation of neuronal maturation capacity during CNS maturation has been correlated with a decrease in the neurite outgrowth promoting properties of astrocytes and, in vitro, the ability of neonatal astrocytes to promote neurite outgrowth is dependent on the length of time in culture (Smith, G. M. et al., 1990. Dev. Biol. 138:377). Neurite outgrowth on astrocytes has been linked to the expression of cell surface molecules such as NCAM, laminin and N-adhesion. Changes in the neurite outgrowth promoting ability of astrocytes can be expected to occur in step with changes in levels of these cell surface molecules. We have investigated this phenomenon by measuring neurite growth-promotion of NCAM and HNK-1 in astrocytes as they mature during culture in vitro.

A time course study of neurite outgrowth from embryonic chick optic nerve ganglion cells on neonatal and in vitro-aged astrocytes showed that a 12h outgrowth period was optimal for detecting differences between them. Neurite outgrowth on neonatal cortical astrocytes was maximal at 2 days of culture and progressively declined, generally beginning to level off on astrocytes cultured for 6 days in vitro. NCAM and HNK-1 expression of the astrocytes was measured by flow cytometry. HNK-1 expression was maximal at 2 days of culture while NCAM reached peak levels at 4 days. Both NCAM-1 and NCAM declined to low levels by 14 days.

These temperature dependent changes in vitro in neurite outgrowth promotion and expression of NCAM and HNK-1 have been correlated with the critical period for regeneration during development in vivo (Smith, G. M. et al., 1986. J. Comp. Neurol. 251:23).

456.6 CALCIUM WAVES PROPAGATE VIA GAP JUNCTIONS IN GLIOMA CELLS TRANSFECTED WITH CONNEXIN 43. A. Charles, C. Naus*, D. Zhu*, E. Dirksen* and M. Sanderson*.
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C6 glioma cells in culture express low levels of connexin43 and have correspondingly weak gap junctional communication as evidenced by dye coupling. These cells have been transfected with the cDNA encoding connexin43, resulting in clones which exhibit different levels of connexin43 expression and dye coupling, and decreased proliferation. We have previously reported that decreased gap junctional communication of a single cell in mixed glioma or purified astrocyte culture induces a wave of increased [Ca2+], that is communicated from the stimulated cell to surrounding cells. In C6 glioma cells, mechanical stimulation induced a Ca2+ wave that was either not communicated or spread only to 1 or 2 adjacent cells. By contrast, C6 cells transfected with connexin43 cDNA showed extensive intercellular propagation of Ca2+ waves, with the distance and rate of intercellular propagation in different clones correlating directly with the level of connexin expression. A clone showing moderate expression of connexin43 showed propagation of Ca2+ waves to 5-10 adjacent cells, whereas a clone showing greater expression of connexin showed propagation of Ca2+ waves to 10-40 adjacent cells. These results provide direct evidence that Ca2+ waves are propagated intercellularly via gap junctions. Since transfection with connexin43 has been shown to reduce proliferation of C6 cells, intercellular Ca2+ signaling may participate in the regulation of cell proliferation.

456.7 PROTEIN PHOSPHORYLATION IN HYPOOSMOTIC-TREATED ASTROCYTES: JT Neary, AS Bender, J. Blighanks* and MD Norenberg.

Calcium appears to play an important role in hypoxically-induced swelling and regulatory volume decrease (RVD). Previous studies from our laboratory using inhibitors of calcium-dependent protein kinases (Bender et al., 1990) suggested that the volume sensitivity of calcium may be mediated by protein phosphorylation systems. To test this possibility, we studied 32P incorporation in intact astrocytes during hypoxically-induced swelling and RVD. Because astrocytes have a high intracellular ATP (100 μM, 1 min), which also increases intracellular calcium in astrocytes, causes a marked dephosphorylation of these proteins. We also found that dephosphorylation of 24 and 21 kDa proteins did not occur in a swelling model which does not undergo volume regulation (exposure to 80 mM external potassium). Taken together, our studies indicate that calcium-dependent protein phosphorylation/dephosphorylation systems play a role in hypoxically-induced swelling and volume regulation.

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Histopathological features of ALS are commonly described as the isolation and sequestration of upper and lower motor neurons and a pronounced demyelination in the primary motor tracts. We have discovered astrogliosis in ALS uniquely within the subcortical white matter. In all cases of ALS examined, the glial fibrillary acidic protein (GFAP) positive astrocytes have the morphological parameters of an on-going, "reactive" process and 2) the astrogliosis is widespread, as it is observed in multiple cortical areas, the midfrontal, inferior parietal, temporal, cingulate, and occipital cortices, as well as the motor cortex. To address the issue of neuronal degeneration and astrogliosis directly, we developed Marchi staining on cryosections and compared Marchi degeneration products in parallel with GFAP immunoreactivity. The amount of astrogliosis did not correlate with Marchi products. ALS was compared to eight different neurological diseases; the gliosis was similar to that in other diseases with gliosis (p < .001). Cytologically, ALS astroglia resemble those present in cases of cerebral infarction, similar to that in other diseases with gliosis (p < .001). Cytologically, described astrogliosis in ALS was most prominent in the subcortical white matter and its astrocytes as a special region of involvement.
456.9
FETAL GLIAL CONDITIONED MEDIUM STIMULATES THE PROLIFERATION AND DIFFERENTIATION IN VITRO OF TYPE I AND TYPE II ASTROCYTES ISOLATED FROM ADULT SPINAL CORD. L. Webster, A. Tessler, P. Levy. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

We are currently investigating the processes involved in the growth and differentiation of astrocytes derived from adult spinal cord in an effort to determine ways of modifying the environment to enhance axon growth after injury. Our previous study demonstrated that adult astrocytes do not survive well in the adult spinal cord, but survive well when they are grown in co-culture with E18 astrocytes and allowed to share medium but prohibited from direct contact. In order to determine the effects of fetal glial cells on astrocyte proliferation, astrocytes derived from adult spinal cord, grown in co-culture with E18 astrocytes, are capable of dividing and differentiating to produce GFAP-positive cells with distinct type I and type II morphologies. Cells of connective tissue origin and PDGF do not have a similar capability to stimulate adult glial growth. It is likely that the E18 astrocytes are secreting a factor(s) that is capable of promoting the division of both quiescent type I astrocytes and O6A progenitor cells. Other factors known to stimulate glial growth are currently being tested for proliferative stimulating activity. Supported by NIMH Research Service, USAMRDC grant 5103502, and NIH grant NS24707.

456.11
ELECTRICAL PROPERTIES OF CULTURED MOUSE ASTROCYTES AFTER BARBITURATE EXPOSURE RESEMBLE PROPERTIES OF RAT ASTROCYTES. N.S. Mogos*, A. Williams. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, 57N OWO, Canada.

The issue of cultured mouse astrocytes having a 20-fold or higher channel-mediated K+ turnover than cultured rat astrocytes continues to be addressed. Electrophysiological studies to date have used rat astrocytes almost exclusively, with many authors being unaware of this difference and ignoring its importance in interpreting results. The membrane potential and input resistance of type I-like cortical astrocytes, cultured from newborn mice, were recorded with and without barbituric (1 mM) exposure. Barium decreased the membrane response to 0 and 35mM K+. It also revealed a Na+K+ATPase mediated transient component, as evident by a biphasic depolarization to ouabain (10mM), which was monophasic without barbituric. GABA or glutamate elicited small, monophasic depolarizations in untreated astrocytes. Barium-treated astrocytes showed further transient responses to either transmitter, similar to rat astrocyte responses as reported in the literature. Uptake blockers for either transmitter responses. The low channel-mediated K+ flux in cultured rat astrocytes must be considered when using this preparation as a model of astrocyte function.

456.12

Cultures of rat cerebral astrocytes were treated with ganglioside GM1 (30 μM) for 22 h, followed by ganglioside-free medium (2 h) or GM1 (30 μM, 2 h). The distribution of GM1 in the astrocyte membrane was then evaluated by indirect immunofluorescence using the B-subunit of cholera toxin (BCT). Approximately 60% of the astrocytes showed BCT-binding, with considerable variability across cells. After treatment with GM1, 70% of the astrocytes showed BCT-binding. In cultures where exogenous GM1 was previously withdrawn, BCT bound predominantly to the outer portions of the cells. These results suggest that the conformation or distribution of GM1 in the astrocyte membrane changes upon withdrawal of exogenous GM1.

456.13
MODULATION OF EPIDERMAL GROWTH FACTOR BINDING IN ASTROCYTES BY HORMONE AND PHOSPHORYLATION INFLUENCES. K. Huff and H. Tang*. Harbor/UCLA Med Ctr & Childrens Hospital, Los Angeles, CA 90029.

The regulation of EGF binding including trans- mechanisms of the receptor is multifactorial in many systems but not well understood in astrocytes where the majority of the CNS binding sites reside. We have studied 125-l labelled ligand binding in cultured neonatal astrocytes after 48 hr stabilization in serum free media. Increase in binding was a rapid response to elevators of intracellular cAMP, inhibitors of protein kinase A, and to insulin, which have cell membrane sites of action. Protein kinase C inhibitors partially reversed PMA and growth hormone down regulation. Decrease in binding was seen as a slower response to deximethasone, and no response in binding was seen to dibutyryl cAMP. Though this hormone produced other effects. Regulation of receptor ligand effects may occur through a common pathway in addition to transmodulation by other growth factors.
456.15
DELAYED RECTIFIER CURRENTS EXPRESSED BY CULTURED RAT MICROGLIA. A. R. Koester and C. G. Nitsch. Department of Psychology, University of Wisconsin, Madison, WI 53706.

Microglia are thought to be the resident macrophages of the brain. Because many functions of immune cells, such as interleukin-1 production by macrophages, appear to require K⁺ channel expression, we have recorded from cultured rat microglia using whole-cell patch clamp techniques. Two types of voltage-gated K⁺ currents were expressed: a previously described inward rectifier current (Iᵣ) and a delayed rectifier current (Iᵳ). The inward rectifier current activates at potentials positive to -40 mV, and the rates of activation and deactivation are voltage-dependent. The tail currents for Iᵣ reverse close to the expected E₉₀ in both 4.5 mM and 100 mM extracellular K⁺. TEA (10 mM) reduces the magnitude of Iᵣ. The rate of inactivation of Iᵣ during a sustained depolarizing pulse varies from cell to cell. Many cells displayed slow recovery from inactivation, as reflected by the amount of use dependence of Iᵣ. Iᵣ is therefore similar to delayed rectifier currents described in many preparations.

456.16
TRANSPLANTATION OF PURIFIED GLIAL CELL POPULATIONS INTO THE MYELIN DEFICIENT RAT. J. D. Duncan, C. Palmen, F. R. Noell, D. R. Archer, and J. D. Duncan, School of Veterinary Medicine, University of Wisconsin, Madison, WI and The Miami Project, University of Miami, Miami, FL.

Previous studies on glial cell transplantations into the CNS of the myelin deficient (md) rat have shown that transplanted glial cell suspensions from neonatal or adult rats can myelinate many axons in the area of the injection site. When the spinal cord specimen produced by the grafted cells was derived from oligodendrocytes that were mature at the time of transplantation or from progenitors that was not critically evaluated. The present study was designed to compare the capacity of mature oligodendrocytes and progenitors to produce myelin in the md rat. Progenitor cells from normal, neonatal littermates labelled with antibody in 129, or mature oligodendrocytes from normal adults labelled with antibody to 01 were purified by fluorescence activated cell sorting. The final purity of sorted cells was 95% or greater. Following sorting, 75,000 - 100,000 cells were injected into the spinal cord of 2-3 day old md rats and the rats perfused at 14-20 days later. A number of rats were injected with freshly dissociated, non-sorted glial cell suspensions from adult rats. Patches of myelin were seen after injection of either progenitor or adult oligodendrocytes; however, the greatest amount of myelin was consistently seen in the rats injected with unsorted cells. These results show that: 1) transplanted 1295 positive progenitor cells, purified by cell sorting, are capable of generating myelinating oligodendrocytes, and 2) mature oligodendrocytes from adult animals are capable of producing md rat axons. (Supported by NIMH grants, RG-219-A-1 and RG-1990-A-3.)

456.17
MYELINATION IN THE MYELIN DEFICIENT RAT BY TRANSPLANTED CANINE GLIAL CELLS. D. B. Archer*, R. Hoffman*, V. Miletic, and I. P. Duncan, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

Studies of allografts of mixed glial cell suspensions injected into the CNS of the md rat have shown that transplanted glial cells are capable of myelinating axons. In this study we examined the capacity of canine glial cells to myelinate host axons following transplantation into the md rat CNS. Mixed glial cell suspensions were dissociated from the spinal cord of a seven week old dog. After 24 hours in culture approximately 100,000 cells were injected into the T13/L1 region of the md rat spinal cord. Fifteen days later the animals were euthanized by perfusion fixation and the transplant site examined by light and electron microscopics. Patches of myelination attributable to transplanted oligodendrocytes were seen in the dorsal columns of the graft recipients. In many preparations, some regions of transplanted cells were myelinated with unsorted cells. These results show that: 1) transplanted 1295 positive progenitor cells, purified by cell sorting, are capable of generating myelinating oligodendrocytes, and 2) mature oligodendrocytes from adult animals are capable of producing md rat axons. (Supported by NIH grants, 1R01 NS26346-01A1 and 1R01 NS22132-01A1.)

456.18

Oligodendrocytes (OL) from adult rats do not form myelin within two weeks after transplantation into juvenile md spinal cord, while fetal glial cells do (D. Neurosci Res 26:278). One possible cause is a host immune response directed against donor OL antigens not expressed by precursor cells. To test this, we cultured adult (P32-55) or fetal (E15-16) rat OL, labeled them with fast blue and transplanted them into spinal cords of P6-9 rats treated with cyclosporine (10mg/kg/day). 11-14 days later, md that had received adult transplants showed fast blue and macrophages within the dorsal columns, but still no significant myelin formation. In contrast, fetal donor cells, lacking mature (GC+) OL at the time of transplantation, formed large islands of myelin that exhibited normal periodicity and formed normal axo-glial junctions. The results do not support an immune-mediated mechanism for the failure of transplanted adult OL to form myelin and are consistent with the view that immature rather than mature cells are needed for initiation of myelination. Supp. by NIH & NIMH.

456.19
A DIFFERENTIAL SCREENING APPROACH TO ISOLATE NEW OLIGODENDROCYTE-SPECIFIC cDNA CLONES. N. Schroeder, N. Nakamura, C. Schadé, G. Yangopoulos and M.E. Schwab. Brain Research Institute, University of Zurich, August-Forster-Strasse 1, CH-8020 Zurich/Switzerland. 2 Regeneron Pharmaceuticals, Tarrytown, N.Y.

Oligodendrocytes are a very special and important cell type, both, from a functional and from a cell biological point of view. In addition to forming myelin they also express specific proteins inhibiting neurite growth. To identify and characterize these proteins, a postnatal P16 rat spinal cord cDNA library was differentially screened with either probes from first strand cDNA synthesis of P16 spinal cord (plus probe) or probes from X-Ray-treated P16 spinal cord (minus probe). Twelve probes were identified by 5'- and 3'-P mapping and the NCE of six were identified by sequence analysis. Two probes were cloned using PCR and then aligned by DNA sequencing. Clone 108-4 was shown to be a novel protein. The human homologue, which shares a high degree of amino acid sequence similarity with the mouse and rat homologues, is expressed in the early postnatal period and is present on oligodendrocytes and Schwann cells. The clone 108-4 is a novel marker for oligodendrocyte differentiation. The clone 108-4 is a novel marker for oligodendrocyte differentiation.
457.1

VACCINA VIRUS-MEDIATED EXPRESSIO N OF PLASMA MEMBRANE TRANSPORTERS: FUNCTIONAL ANALYSIS OF THE CATECHOL-O-METHYLTRANSFERASE (COMT) ENZYME.

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1Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD
2Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD

We have characterized a novel vaccinia-virus based transient expression method suitable for the rapid functional characterization of neurotransmitter transporters and this system to an analysis of the activity of the cloned human MAO-N-l-phenylethylamine. The expression system utilizes a vaccinia-virus based expression system, in infected cells, cytoplasmic transcripts from transfected plasmids bearing T7 RNA polymerase. To characterize this system, we transfected separately the intestinal Na+/glucose and brain Na+/GABA transporters into vaccinia-expressing cells. Using a molecularly cloned cDNA encoding the human Na+/GABA-transporter (cDNA), we applied this system to document the functional properties of the expressed transporter in HeLa fibroblasts. Following transfection with NET, vaccinia-T7 infected cells expressed saturable (Kd=457 nM) Na+ dependent uptake of [3H]L-norepinephrine which is antagonized by antidepressants (desipramine, mirtazapine, imipramine). D-amphetamine (Kd=457 nM) and cocaine (Kd=140 nM). G3H 12909 and paroxetine exhibited weak potency (Kp=100 nM) relative to their actions on brain dopamine and serotonin transporters, respectively. Analogists of adrenergic receptors, other plasma membrane transporters, and vesicular amine transporter failed to block NET activity. Further work is underway to characterize and localize the direct binding of radiolabeled antidepressants to the cloned carrier. These results demonstrate that a single cDNA encodes many if not all of the readily observable properties of the high affinity, human norepinephrine uptake system.

457.2


Dept. of Mol. Pharma & Tox., Sch. of Pharm., Univ. of South. Calif., L.A., CA 90033

Monoamine oxidase (MAO) A and B oxidize a number of neuroactive amines and they are coded by separate genes (PNAS:85,4934,1988). Recently, we have isolated genes encoding human MAO-A and B by screening four bacteriophage libraries with human MAO-A and B cDNA probes. Twenty-one MAO-A positive clones and forty-three MAO-B positive clones were isolated. Southern blot analysis of phase DNA isolated from these banded clones showed that one clone hybridized to 

457.3

THE ROLE OF SPICE SITE-ENCODED AMINO ACIDS ON THE SUBSTRATE AND INHIBITOR SENSITIVITIES OF MAO. H.F. Wu, K. Chen* and J.C. Shih*, Dept. of Mol. Pharm. & Tox., Sch. of Pharm., Univ. of South Calif., L.A., CA 90033

We have recently isolated human MAO-A and B genes and showed that they both consist of 15 exons and exhibit identical exon-intron organization. When the splice site-encoded amino acids in MAO-A are compared with those in MAO-B, 11 of 14 amino acids are encoded at the 5' boundary and 9 of 11 amino acids are encoded at the 3' introns, and are identical. Among the different ones only 5 amino acids are not conserved. Thus, we made five MAO-B mutants in which the splice site encoded amino acids were mutated to the corresponding amino acid in MAO-B. These MAO-B mutants were prepared by oligonucleotide-directed mutagenesis. Each mutagenic clone was sequenced then cloned into an expression vector, pcEE. High efficiency CaPO4 precipitation transient transfection was performed. The amount of MAO-B mutant transfected was determined by Western blotting. The substrate specificities were determined by using 3H-serotonin or 14C-phenylethylamine radioassay. The sensitivities to clorgyline or deprenyl was also determined. The role of each of these amino acids on the catalytic properties of MAO-B will be discussed. (Supported by NIH grants R37 MH39085 (Merit Award), K05 MH00776, R01 MH37020 and Welin professorship).

457.4

MOLECULAR ANALYSIS OF THE CATECHOL-O-METHYLTRANSFERASE GENE IN FISCHER AND WISTAR-FURTH STRAINS OF RAT. M.H. Grossman, L.J. Littleell1, and R. Weiss

1Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD

We have characterized a novel vaccinia-virus based transient expression method suitable for the rapid functional characterization of neurotransmitter transporters and this system to an analysis of the activity of the cloned human MAO-N-l-phenylethylamine. The expression system utilizes a vaccinia-virus based expression system, in infected cells, cytoplasmic transcripts from transfected plasmids bearing T7 RNA polymerase. To characterize this system, we transfected separately the intestinal Na+/glucose and brain Na+/GABA transporters into vaccinia-expressing cells. Using a molecularly cloned cDNA encoding the human Na+/GABA-transporter (cDNA), we applied this system to document the functional properties of the expressed transporter in HeLa fibroblasts. Following transfection with NET, vaccinia-T7 infected cells expressed saturable (Kd=457 nM) Na+ dependent uptake of [3H]L-norepinephrine which is antagonized by antidepressants (desipramine, mirtazapine, imipramine). D-amphetamine (Kd=457 nM) and cocaine (Kd=140 nM). G3H 12909 and paroxetine exhibited weak potency (Kp=100 nM) relative to their actions on brain dopamine and serotonin transporters, respectively. Analogists of adrenergic receptors, other plasma membrane transporters, and vesicular amine transporter failed to block NET activity. Further work is underway to characterize and localize the direct binding of radiolabeled antidepressants to the cloned carrier. These results demonstrate that a single cDNA encodes many if not all of the readily observable properties of the high affinity, human norepinephrine uptake system.

457.5

NITRIC OXIDE SYNTHASE: MOLECULAR CLONING OF THE populations in the brain and periphery. Macrophages generate NO to elicit their probe of the cerebellar NOS gene, low stringency screening of a phage library of hybridization techniques were used to isolate the macrophage NOS. Using a molecularly cloned (TIPS 12:125,1991). With this sequence data, various cells of the central nervous system. The goal of this work is to sequence the gene from the form found in brain. These macrophages probably include the microglial

457.6


3-hydroxyanthranilate oxygenase (3-HAO) is the enzyme responsible for the synthesis of quinolinic acid (Quin) in the mammalian brain. An endogenous excitotoxin, Quin, has been postulated to be involved in several neurodegenerative disorders including Huntington's disease (HD). A candidate cDNA encoding a 3-HAO-like enzyme was isolated from a rat liver library using a polyclonal antibody, has been previously reported (Neurosci. Abs., 1998). Here we report the further characterization of this clone and others obtained from cDNA and genomic libraries. Northern blot analysis has revealed that the 3-HAO mRNA is approx. 2.2 kb in rat and mouse and about 1.7 kb in baboon. 3-HAO mRNA is easily detected in kidney and liver total RNA, but not in whole brain RNA. The 3-HAO cDNA clones can be amplified from total brain RNA using PCR. The rat cDNA clone contains an inverted repeat at its 5' end which has the potential to form a 100 bp stem and a 106 bp loop. This 350 bp sequence has been shown to exist on 4 exons in a mouse cosmide. Sequence analysis of the genomic fragment containing the most 5' exon has identified a potential splice acceptor site. 3-HAO cDNA sequences show some homology to the human form but not to the mouse form. Cross-species hybridization on somatic cell hybrids, has localized the candidate 3-HAO gene to human chromosome 17q. A Sprague mouse cross allowed us to map this genomic clone to chromosome 4p.

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457.7

EXPRESSION, ORGANIZATION AND LOCALIZATION OF THE HUMAN N-CHIMAERIN GENE.

Southgate, C. A. S., N. Spurr, C. L. Lim*.

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The novel brain-specific cDNA, n-chimaerin, encodes a predicted product with significant similarities to both the Cbl cysteine-rich motif CXXC,C-X,CX,C of protein kinase C and the product of the imprinted cluster region gene involved in Philadelphia chromosome translocation. The N-terminal half of n-chimaerin shares almost 50% identity with corresponding sequences in the regulatory domain of PKC. The C-terminal half of n-chimaerin has 42% identity with residues 1050 to 1225 of BCR n-chimaerin RNA is specifically expressed in the brain with highest abundance in the hippocampus and the cerebral cortex. The RNA expression pattern is neuronal. The human n-chimaerin gene was localised to a specific chromosome by analysis of somatic cell hybrid DNA and a n-chimaerin related loci was detected on a different specific chromosome. Genomic clones containing the n-chimaerin gene show that three exon boundaries in the n-chimaerin gene precisely correspond to those in the human BCR gene. A further exon boundary corresponded to a (Cys/Pro) protein kinase C splice junction, in the region encoding the cysteine-rich zinc finger motif. The opposite end of the same n-chimaerin exon contains a region of sequence similarity to BCR.

457.9

A VERSATILE CLONING AND EXPRESSION SYSTEM TO ANALYZE FUNCTIONAL PROPERTIES OF HUMAN D2 RECEPTOR ALLELES.

G. M. Lawless1, C. H. J. Ruppers1, T. Kisch2, E. P. Noble1,3, and A. J. Tobin1,3.

Department of Biology, Molecular Biology Institute, University of California, Los Angeles, CA 90024

Dopamine receptors are widely studied because of their role in many neurophysiological disorders. A RFLP in the D2 receptor gene has been found to be associated with patients suffering from severe alcoholism (JAMA, 263:2055-2060). To analyze the functional properties of D2 receptors of different alleles we have developed a PCR-based method to clone and express specific cDNAs. Total RNA is purified from limited amounts of postmortem brain samples, and D2 receptor mRNA is transcribed into cDNA utilizing human D2 receptor specific primers. Two sets of primers allow us to clone the complete open reading frame of receptor sequence after amplification in a thermal cycler. Expression of the cloned sequence in a cell culture system will enable us to analyze and compare the biochemical and pharmacological properties of D2 receptor derived from the two human alleles A1 and A2 for its possible involvement in the appearance of severe alcoholism. (Supported in part by The Seaver Foundation.)

457.11

CLONING AND EXPRESSION OF THE PHOSPHATE ACTIVATED GLUTAMINASE GENE FROM RAT BRAIN.

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Glutamate (Glu) is the principal excitatory neurotransmitter in the vertebrate CNS. One of the primary enzymes involved in the metabolism of Glu is phosphate activated glutaminase (PAG). Using a previously cloned partial cDNA to PAG (PAG1, Benner et al., 1988) as a probe, we isolated a PAG cDNA clone 4 Kb in length (OGA4K). This clone contains all of the sequence previously described in PAG1 as well as an additional 57 bases of coding sequence on the 5' end and 2.7 Kb of 3' untranslated region which includes the polyadenylation signal. Antisense riboprobes generated from this clone were used in in situ hybridisation assays to study the expression of PAG RNA in rat cardiac and skeletal muscle, lung, kidney, intestine, liver and brain. PAG expression was low in all of these cell lines and was primary astrocytes and granule cells. A genomic clone for the rat PAG gene was isolated through homology to the OGA4K cDNA clone. Partial characterisation of this clone has demonstrated that it extends to the 5' end of the gene, and in situ exons extends into the coding region of the gene. The two exons terminate in consensus sequences for splice sites.

457.12

GENES FOR RAT GLUTAMIC ACID DECARBOXYLASES.


Department of Biology, Brain Research Institute, and Molecular Biology Institute, University of California, Los Angeles, CA 90024

The brains of rats and humans contain at least two forms of glutamate decarboxylase (GAD65 and GAD67), which differ in sequence and size, and which derive from different genes. The human GAD65 gene contains two promoters whose usage differs between adult and embryonic brain. We have isolated a rat genomic clone for GAD65 and GAD67. The restriction maps of these cloned DNAs suggest that the rat GAD65 gene spans a length of approximately 34 kb and the rat GAD67 approximately 32 kb. The two rat GAD genes derive from different genomic clones: GAD67 cDNA hybridizes to genomic restriction fragments distinct from those that hybridize to GAD65 cDNA and GAD65 does not hybridize to GAD67 genomic clones. The sequence of the putative promoter region of the rat GAD67 gene resembles, in several respects, that of the corresponding region of the human GAD65A gene. Functional analyses are now underway to determine the molecular basis of the coexpression and the independent regulation of the two GADs. (Supported by NIH grants NS 20356 and NS 22256)

Using previously described fetal and rat cDNAs, we isolated human GAD 

3 and GAD 

4 cDNAs that contain complete open reading frames. GAD 

3 cDNA encodes a polypeptide of 585 amino acids, while GAD 

4 cDNA a polypeptide of 596 amino acid residues. Northern blot analysis of a single RNA species for each GAD using extracts of both fetal and adult human brain, with GAD 

3 mRNA 5.7 kb long, and GAD 

4 3.7 kb long. Sequence analysis shows high conservation of each coding sequence between rats and humans, about 90% at the nucleotide level and 96% at the amino acid level. To obtain human GAD 

3 and GAD 

4 protein from recombinant DNA, we cloned the coding region of human GAD 

3 and GAD 

4 cDNA into bacterial expression vectors and expressed enzymatically active protein in E. coli. Using human GAD 

3 and GAD 

4 genomic clones, we determined the chromosomal localization of the two GAD genes by in situ hybridization. (Supported by NIH grants NS03036 & NS22256)

SYNAPTIC STRUCTURE AND FUNCTION 1

PROTEIN METHYLATION IN SYNAPTOSOMES. I.S. Wright* and F.I. Siegel. Deps. of Pediatrics and Physiological Chemistry, Univ. of Wisconsin, Madison, WI 53706. Synaptosomes from five regions of adult rat brain (cortex, cerebellum, hippocampus, brain stem and striatum) and three regions of 2 day old rat brain (cerebellum, brain stem and remaining brain) were isolated and used in studies of protein methylation. Synaptosomes were incubated with [methyl-3 H]S-adenosyl-L-methionine at pH 6.5 and at pH 8, and methylated proteins were visualized by SDS-PAGE and fluorography. For adult brain, methylated profiles in synaptosomes were compared with those in P, P, P, and cytosolic fractions. At pH 8, a protein of 48kDa was methylated synaptosomes from all brain regions to a greater extent than in other subcellular fractions, and in cerebellar synaptosomes a protein of 56kDa was a major methyl acceptor protein. Neither of these proteins was methyalted at pH 6.5, indicating that this is not aspartate carbamoylation. In synaptosomes from the two day old rat brain, methylation of 68kDa and 48kDa (all brain regions), but not 56kDa was found. A methyl acceptor protein of 35kDa was found in all fractions and a family of 22kDa-36kDa methylated proteins was present in all membrane fractions. These results indicate an age-dependent, region specific methylation of synaptosomal proteins. (Supported by NIH grants NS24969, GM38497 and HD03352.)

SYNAPTIC STRUCTURE AND FUNCTION 1

AUTOPHOSPHORYLATION OF CEREBELLAR AND FOREBRAIN FORMS OF CAM-KINASE-Gr. O.B. McDonald and N. Sanyoun. Wellcome Research Laboratories, 3030 Cornwallis Road, RTP, NC 27709.

A neuronal Ca- and calmodulin-dependent protein kinase, CaM

Kinase-Gr, occurs as Mr 65,000 and 67,000 polypeptides in the rat cerebellum and as Mr 65,000 polypeptide in the rat forebrain. Both enzyme forms were purified and found to undergo stoichiometric autoprophosphorylation on a serine residue. Autophosphorylation cause a characteristic decrease in the electrophoretic mobility of both polypeptides. This change in gel mobility was completely reversed by the action of an endogenous brain Mr 70,000 phosphoprotein phosphatase, or by the addition of an acid phosphatase. The shifts in gel mobility were confirmed by silver staining, autoradiography and immunoblotting with a CaM Kinase-Gr antisemur. Autoprophosphorylation of the cerebellar and forebrain enzymes, acquired an autonomous, Ca- and calmodulin-independent activity, and displayed greatly increased sensitivity to subsequent exposure to Ca- and calmodulin.
548.5 TWO LOCI WITH EXTENSIVE POLYMORPHISM ENCODE SNAP-25 IN GOLDFISH. C. Risinger and D. Larhammar, Dept of Medical Genetics, Roskilde University, S-4000 Roskilde, Denmark. SNAP-25 (synaptosome-associated protein) is a 25-kDa protein which is expressed exclusively by neurons. It is localized to presynaptic nerve terminals and is associated with synaptosomal membranes. Its expression starts at the time of synaptogenesis. We have previously shown that SNAP-25 is an extremely well-conserved protein with implications important functions. The chicken and mouse SNAP-25 proteins are identical throughout the 206 amino acids (S. Catsicas et al., PNAS 88, 785-789, 1991), and SNAP of goldfish, ray (Torpedo marmorata), river lamprey, and Drosophila melanogaster show extensive similarity to the mouse protein (Abstract 154-14, Neuroscience Meeting 1990).

Surprisingly, all six clones that we have isolated from a goldfish retina cDNA library are distinct at the nucleotide level. They can be sorted into two groups according to degree of sequence identity. Within each group, the clones are virtually identical at the amino acid level. Between the two groups there is only 90% amino acid sequence identity. Furthermore, some clones contain mutations which preclude synthesis of a functional SNAP-25 protein. Our results suggest that there are two loci for SNAP in goldfish in agreement with chromosome analyses which have shown that goldfish are tetratoploid. The gene duplication has presumably allowed the redundant gene copy to accumulate deleterious mutations.

Our findings of SNAP gene duplication and polymorphism suggest that other cloning results obtained in goldfish should be regarded with caution until multiple clones have been analysed.

548.6 GENE ORGANIZATION OF SYNAPTIC PROTEIN SNAP-25 IN DROSOPHILA. I. Lundin, C. Risinger, A. G. Broomer, C. Bank, and D. Larhammar, Dept of Medical Genetics, Uppsala University, S-751 23 Uppsala, Sweden.

SNAP-25 is a 25-kDa protein which is expressed exclusively by neurons. It is associated with the inner side of the cell membrane in presynaptic nerve terminals. The expression of SNAP-25 starts concomitantly with synaptogenesis, indicating a role in this process or in the functions of the mature synapse.

We have previously shown that the chicken and mouse SNAP-25 proteins are identical throughout the 206 amino acids (S. Catsicas et al., PNAS 88, 785-789, 1991) and that SNAP of goldfish and Torpedo show approximately 90% similarity with the mouse sequence (see abstract by C. R. and D. L.). Using low-stringency hybridizations, we have isolated SNAP-25 cDNA clones from a Drosophila melanogaster library. Drosophila SNAP-25 displays 80% sequence identity to the chicken, and a segment of 60 amino acids shows 85% sequence identity. We have also isolated several genomic SNAP-25 clones from a phage library. Determination of the sequence of deafened flies shows that the Drosophila SNAP-25 gene structure is as complex as that of the chicken SNAP-25 gene which consists of eight exons spanning more than 65 kbp.

These studies show that SNAP-25 is one of the most highly conserved synaptic proteins known which suggests a crucial role for SNAP-25 in synaptic function.
458.9

ISOLATION AND PRELIMINARY CHARACTERIZATION OF SYNAPTIC VESICLE-PLASMA MEMBRANE COMPLEXES ('ACTIVE ZONES'). C. Walch-Sukowa* and M. A. Jahn. Max-Planck Institute for Biophysical Chemistry, Am Klopferspitz 18a, D-38033 Göttingen, Germany.

At present, little is known about the molecular constituents of the contact sites between synaptic vesicles and the plasma membrane ('active zones') in nerve terminals. Here, we report the isolation and preliminary characterization of putative active zones from adult rat brain. The isolation involved purification and lysis of nerve terminals, followed by separation of membrane-bound from free synaptic vesicles and finally immunoisolated material to a bead matrix coated with monoclonal antibodies against synaptic vesicle proteins. Electron microscopic analysis revealed that the adsorbed material contains synaptic vesicles attached to membrane fragments, resembling the pre- and post-synaptic membranes. Using immunoblotting, a co-enrichment of synaptophysin and synaptotagmin, two membrane proteins of synaptic vesicles, with the immunoisolated material was confirmed by SDS-polyacrylamide electrophoresis revealed the presence of membrane proteins different from those found in purified synaptic vesicles. A comprehensive analysis of the protein content is currently in progress.

458.10


A monoclonal antibody, B16 (sub-type IgM) has been produced that recognizes a protein with molecular weight of 88,000 daltons throughout the brain and retina. Within the retina the antigen is concentrated at the ribbon synaptic structure in the photoreceptor terminal. We have found that in every species in which we have examined (fish, frog, lizard, mouse, rat, rabbit, cat, cow, monkey), we are presently purifying the antigen with two aims in mind: 1) the purified protein will be used as antigen to produce a panel of IgG monoclonal antibodies to the orginal protein. 2) the purified protein can be partially sequenced with the intent of producing primers for PCR. We have purified the protein using differential centrifugation, IEF column chromatography, and SDS-PAGE. The protein is fairly soluble, requiring 70% salt to precipitate. Thus, with differential centrifugation, we have been able to purify the B16 protein by 30-40 fold. This semi-purified fraction was then applied to an Iso-Electric-Focusing column (IEF) and focused for 48 hours. The fraction containing the B16 protein eluted at roughly pH 7.3 +/- 0.2 and resulted in a 3,600 fold purification. The 7.3 pH fraction was then applied to a 5-15% gradient SDS-PAGE and Western blotting. The 88kDa band was cut out of the blot and the protein eluted via 1% SDS-1% Triton. The purity is 100% as determined by initial amino acid analysis. From 100 grams of brain we have recovered 50 ug of purified protein. (Supported by the NSF BNS-8919829 and the Boston College Research Fund.)

458.11

DETECTION OF DYSTROPHIN IN A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM ADULT RAT BRAIN. Kim T.W., M. C. Englund, L. D. Sheppard, M. A. Capaldi, J. A. Black. 1. Graduate program in Physiol. and Neurobiol., Rutgers-The State U of NJ and UMDNJ/Robert Wood Johnson Medical School, Dept. of Neurosci., UMDNJ/Robert Wood Johnson Medical School, and Rutgers-Newark Medical School, New Brunswick, NJ.

Rutgers-The State University of NJ and UMDNJ/Robert Wood Johnson Medical School of Medicine, New Brunswick, NJ. ABSTRACT: dystrophin, a 1.5-MDa membrane cytoskeletal protein, is found concentrated at the ribbonsynaptic complex and is thought to play a role in synaptic transmission. The overall percentage of dystrophin present in the structure under normal conditions is unknown. We have recently determined that dystrophin was found to be present in brain. Preliminary immunocytochemical analysis has shown that the dystrophin is localized in the PSD. In the present study, we directly demonstrate the presence of dystrophin in the PSD fraction isolated from cerebral cortex (CTX), cerebellum (CBL) and globus pallidus (GP) of adult rat brains. The amount of dystrophin present in the structure was determined by western blot analysis using the antibody, anti-dystrophin. In all PSD fractions examined, the antibody detected multiple proteins with apparent molecular weight higher than 110 kDa under denaturing and reducing conditions. The relative proportion of proteins differed in CTX, CBL and CBL, suggesting differential regional expression. Moreover, in CTX, there was 10-fold greater dystrophin in PSD than in synaptic membranes or total homogenate, confirming previous immunocytochemical localization. We are presently characterizing dystrophin in PSD from developing and adult brains. Our results indicate that dystrophin is a component of the PSD, potentially suggesting abnormalities of dystrophin function at the synapse that underlie cognitive deficits. (Supported by grants from NINDS, NICHD and McKnight Foundation)

458.12


ABSTRACT: The olfactory bulb (OB) contains the initial synapses in the nervous system and is the site of initial differentiation of receptor cells. In order to study the biochemical properties of the OB synapses, we have isolated a PSD from the OB by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424. We used several biochemical methods to characterize the OB-PSD. The PSD was generated by the method of Menas and Compton, 1982, Science 216:422-424.
SYNAPTIC STRUCTURE AND FUNCTION: HIPPOCAMPUS

459.1
ANATOMY OF ELECTROPHYSIOLOGICALLY CHARACTERIZED INTERNEURONS IN IMMATURE HIPPOCAMPUS. C.M. Gómez, E.L. Smith, J.W. Swauger. Dept. of Anatomy, College of Medicine, Albany Medical College, Albany, N.Y. "Interneurons which promote neurotransmitter release are thought to be important for normal synaptic transmission. These cells consist of three classes: interneurons with terminal arbors in stratum pyramidale, interneurons which densely innervate stratum radiatum, and interneurons which have terminal arbors primarily in stratum moleculare. Each of these classes is further divided into subpopulations. In this study, we attempt to identify these subpopulations by analyzing the morphologies of interneurons which can be identified as interneurons when they display brief action potentials with little post-spike hyperpolarization. We recorded from interneurons in the CA1 and CA3 regions of guinea-pig hippocampal slices and characterized them using a combination of extracellular and intracellular recording techniques. Our results suggest that these immature hippocampal interneurons have many of the morphological features commonly ascribed to interneurons in mature hippocampus.

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Interneuronal recordings were obtained from fast spiking cells in the CA1 and CA3 regions of 10-16 day old guinea-pig hippocampal slices. Characteristically, these cells have high firing rates and a prominent afterhyperpolarization. By using whole cell patch clamp techniques we have shown that these cells also possess short tonic postsynaptic potentials, suggesting that they are excitatory interneurons. In addition, these cells also have fast-spiking properties suggesting that they are inhibitory interneurons. Our results suggest that these cells may represent a dualistic population of interneurons that are involved in both excitatory and inhibitory synaptic transmission.

459.3
GLYCOLYSIS IS NECESSARY FOR NORMAL SYNAPTIC TRANSMISSION IN GUINEA-PIG HIPPOCAMPAL SLICES. Peter Lipton. Dept. of Physiology, Albany Medical College, Albany, N.Y. 12208.

The morphology of subpopulations of CA3 hippocampal interneurons were characterized. Neurons were impaled in Stratum pyramidale (S. pyramidale) of slices taken from area CA3 of 1 to 2 week old rat hippocampus. Cells were identified as interneurons when they displayed clear action potentials with prominent afterhyperpolarization and high firing rates in response to depolarizing steps of intracellularly injected current. Biocytin was injected via the recording electrode. Approximately 50% of such labeled single cells, 65% were dye-coupled with the number of cells filled ranging from 2 to 6. Thus far, three classes of interneurons have been identified all of which possess dense basket-like terminal arbors. These include 1) pyramidal basket cells and 2) stellate cells, both with terminal arbors primarily in S. pyramidale; and 3) stellate cells with terminal arbors primarily in stratum moleculare. The dye coupled cells, 10% were coupled to at least one other interneuron. In one instance, 6 interneurons were dye coupled. 30% of cells were coupled to spiny pyramidal cells which characteristically have intrinsic burst properties. The density of terminal arbors and breadth of projection within the CA3 field suggests that these immature hippocampal interneurons have many of the morphological features commonly ascribed to interneurons in mature hippocampus.

459.4
PROPERTIES OF INHIBITORY AND EXCITATORY MINIATURE CURRENTS IN HIPPOCAMPAL NEURONS MAINTAINED IN LOW DENSITY CULTURE. K.S. Wilcox and M.A. Dichter. Dept. of Physiology & Neurology, Univ. of Pennsylvania & Graduate Hospital, Phila., PA. 19104

Electrophysiological properties of inhibitory and excitatory neurotransmission were studied using the whole cell patch clamp technique in hippocampal neurons maintained at an extremely low density in dissociated cell culture. Neurons were prepared as described (Buchallner & Dichter, 1991), plated at 3.5-10^4 cells/ml, and media was supplemented at 1.5 days with 20 mM [K+] media was supplemented at 1.5 days with 20 mM [K+] media was supplemented at 1.5 days with 20 mM [K+] media was supplemented at 1.5 days with 20 mM [K+]. Inhibitory and excitatory spontaneous miniature currents were recorded in the presence of TTX in neurons 2-3 weeks old. Miniature inhibitory currents occurred at lower frequencies than excitatory currents (0.01-0.3 Hz vs 0.05-16 Hz), had lower rates of decay (>10 msec vs <4 msec) that were prolonged with depolarization, were blocked by GABA receptor antagonists and prolonged with pentobarbital. Excitatory currents were blocked by glutamate receptor antagonists and showed NMDA and non-NMDA components. The frequency of currents increased with application of hyperosmotic bath solution. Amplitude distributions exhibited a wide degree of variance for both currents. These results provide evidence that neurotransmission at both types of synapse is quantal. This evidence is essential before quantal analysis can be used to study short term changes in synaptic efficacy. Recordings from isolated pairs of neurons have demonstrated that frequency dependent changes in synaptic strength occur at inhibitory and excitatory synapses in this low density culture system. Supported by NS24260 to M.A.D.
459.5 COMPARISON OF TWO ANATOMICALLY DISTINCT EXCITATORY SYNAPTIC INPUTS TO CA3 PYRAMIDAL NEURONS. S.F. Williams et D. C. Livingston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The two major excitatory synaptic inputs to area CA3 of the hippocampus, the mossy fiber (MF)/CA3 and the commissural/associational fiber (CA/A), exhibit different forms of long term potentiation. This may in part reflect a differential distribution of NMDA receptors on the CA3 neuron's dendritic tree. These synapses are likely to terminate at different levels of the dendritic tree. We have investigated the kinetic properties of these two synaptic inputs. Rat hippocampal slices were maintained at 2-34°C. Inhibition was blocked by 10 μM picrotoxin and 10 μM bicuculline, and Ca2+ and Mg2+ ions were raised to 5 mM to prevent epileptiform activity. Single electrode current- and voltage-clamp recordings were made. The more distal CA/A synapses had a slower 10-90% rise time and a slower decay time constant (τD) than the MF synapses. The τD of the MF EPSC was not voltage dependent. The CA/A synapse did show a voltage-dependent prolongation of synaptic current at depolarized potentials (+20 mV or more). This was blocked by APV. The rise time of the EPSP for the two inputs were similarly different. The τD of the EPSP was also measured. If the dendritic tree is purely passive one would expect the EPSP to decay with the membrane time constant. We observed that both the MF and CA/A EPSPs decayed more slowly than the membrane time constant. This effect was most marked for CA/A EPSPs, which decayed with a time constant 1.8 times slower than the membrane time constant (MF decay was 1.3 times slower). This prolongation was voltage-dependent but not affected by APV. These data suggest that the kinetics of these synaptic inputs are affected both by electrophysiological properties and by gated channels in the dendrites. (Supported by grants MH44475, NS11535, and AFOSR 89-0142.)

459.6 QUANTITATIVE ULTRASTRUCTURAL CHANGES ASSOCIATED WITH REACTIVE SYNAPTOGENESIS IN THE CA1 REGION OF THE HIPPOCAMPUS. H.V. WHEAL, S. PHelps* and J. MITCHELL*. (Supported Brain Research Association). Depts of Physiology & Pharmacology & Human Morphology, Southampton University, S09 3TU, UK.

Following the unilateral kainic acid lesion of the rat hippocampus, the effects of the derinnervation on the distribution of asymmetric synapses in the CA1 pyramidal cell area were quantified. Ultrastructural examination of the proximal, mid and distal sublayers of the s. radix of the hippocampus ipsilateral to kainic acid injection revealed a substantial loss of synapses from these sublayers, which peaked at between 8-10 days post lesion. This was followed by rapid reactive synaptogenesis that was differentially distributed, with the largest number of new synapses concentrated in the sublateral proximal to the CA1 pyramidal cell layer. An interne enhancement of multisynaptic boutons, dendritic shaft and complex synapses was also found in this sublayer.

Measurement of spine parameters revealed significant increases in neck length and width over the same time period. These morphological changes might underlie the significant enhancement of LTP of CA1 CA3 interneuronal coupling in this model.(Turner & Wheal Neurobiology in press)

This project was funded by the Wellcome Trust


The synapse plays a pivotal role in neuronal communication and information processing. Nonetheless, little is known about the factors that regulate synaptic structure. Our previous work indicated that synapses in developing and mature superior cervical ganglion of rats were regulated by presynaptic innervation. In the present study, we investigated whether presynaptic innervation plays a similar regulatory role in hippocampal tissue to define the regulation of synaptic molecular structure in the brain. Lesion of entorhinal cortex, a major source of synaptic input to hippocampus, resulted in a dramatic 31% decrease in calmodulin binding to the novel major postsynaptic density protein, mPSDp, one day postoperatively. The maximum effect, a 55% decrease in mPSDp, was obtained 3 days after denervation. The lesions did not alter total synaptic membrane protein, suggesting that the denervation specifically affected the mPSDp. Combined lesions of fimbria- fornix and the entorhinal cortex resulted in a 66% decrease in the mPSDp one week after the surgery. The present findings suggest that afferent innervation plays an important role in regulating synaptic molecular architecture in the hippocampus, reproducing the findings obtained in the peripheral nervous system.

459.8 MULTIPLE CONTACTS BETWEEN HIPPOCAMPAL CA3 AXONS AND APICAL DENDRITES OF CA1 PYRAMIDAL CELLS. E.E. Sora and K.M. Harris. Program in Neuroscience, Harvard Medical School, & Neurology Research, Children's Hospital, Boston, MA 02115.

Recent findings from quanta analyses of paired recordings between hippocampal CA3 and CA1 pyramidal cells have prompted us to determine the frequency of contacts occurring between these cells (Malinow and Tsien, 1990; Bekkers and Stevens, 1990; Friedlander et al., 1990; Foster and McNamara, 1991). In earlier work (Harris et al., 1989), the rapid Colgi method was used on hippocampal slices from adult rats (30-60 days old) that were maintained in vitro. In the present study, these slices were used to visualize the CA3 axons and the CA1 pyramidal cells. Fifty-five well-imregnated axons were located in s. radiatum between 19 and 370 microns from the apical edge of the CA1 pyramidal cell bodies. 43 of these were from CA3b and 30 from CA3a. All of these CA3 axons traversed the entire width of at least one dendritic arbor from a single CA1 pyramidal cell. A contact was defined as the point where an axon crossed a dendrite touched the dendrite or a dendritic spine in the same focal plane at 1260x magnification. Three classes of contacts were observed: i) single contacts (76%), ii) multiple contacts onto 2 dendrites (17%), 3 dendrites (2%), or 4 dendrites (1%). These data are in accordance with earlier estimates of 1.3 contacts by each CA3 axon (Anderson, 1990, Prog. Brain Res 83:215-222). Thus, at least 24% of the CA3 axons make multiple contacts with dendrites of a single CA1 pyramidal cell, a rate of occurrence that will need to be considered for a complete interpretation of quanta analyses from paired recordings of these cells.

SOCEITY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
460.3 INTERACTIONS BETWEEN ARACHIDONIC ACID AND Ca²⁺ IN THE PHOSPHORYLATION OF GAP-43 (FL, B-50) L.D. Schenck, and J.L. Berwicz, Dept. Neuropsychology, Children's Hospital, Harvard Med. School, Boston, MA 02115

The presynaptic PKC substrate GAP-43 has been implicated in neuronal growth, regulation and plasticity. Ultrastructural hippocampal calcium is characterized by correlated increases in GAP-43 phosphorylation and transmitter releases may depend on the post-synaptic generation of arachidonic acid (AA; 20:4) and its subsequent stimulation of AA-sensitive isoforms of PKC. The present study, synaptosomal membrane from rat central core were used to examine whether AA affects GAP-43 phosphorylation and if it interacts with changes in Ca²⁺ levels. Low concentrations of AA (50 μM) stimulated GAP-43 phosphorylation and that were potentiated by Ca³⁺ (10⁴ M).

460.4 DEPOLARIZATION-DEPENDENT TYROSINE PHOSPHORYLATION IN SYNTAPSOMES. S.J. Woodrow*, N. Bissoon* and J.M. Gurd, Dept. of Biochemistry, Simon Fraser University, Burnaby, BC, Canada; The Rockefeller University, New York.

Protein phosphorylation is involved in the regulation of synaptic activity. Although protein kinase C and protein tyrosine kinases are present at the synapse [eg. Ellis and Gurd (1988) J. Neurochem. 51, 611-620], their relationship to synaptic function is unclear. We have found that depolarization on synaptosomal phosphotyrosine-containing proteins (ptyr-proteins) by immunoblotting with anti-ptyr antibodies. Freshly prepared synaptosomes from rat forebrain contained several ptyr-proteins and these remained essentially unchanged during incubation in vitro. Incubation for 45 min. in the presence of 0.1 M Ca²⁺ followed by depolarization with high K⁺ (41 M) led to an increase in the amount of immunodetectable ptyr associated with a protein of Mr 117,000 (pI 17). This increase was apparent within 1 min. of the start of depolarization, reached a maximum between 3 and 5 min. and then decreased during the next 20 min. Omission of Ca²⁺ from the incubation buffer, or the addition of EGTA 5 min. prior to depolarization prevented the increase in tyrosine phosphorylation of pI 17. The results suggest that the phosphorylation of pI 17 on tyrosine may be involved in the regulation or modulation of synaptic activity. Supported by the Natural Science and Engineering Research Council.

460.5 DEPHOSPHORYLATION OF B-50 (GAP-43) BY TYPE 1 AND 2A PROTEIN KINASES IN RAT SYNTAPSOMAL MEMBRANES. T.Y. Han and L.A. Dobas, Departments of Biochemistry and Molecular Biology and Neurology, Medical College of Ohio, Toledo, OH 43699.

The neuronal protein kinase C substrate B-50 (GAP-43) is dephosphorylated in rat cortical synaptic plasma membranes (SPM) by phosphatase type 1 and 2A (PP-1 and PP-2A). The present studies further demonstrate that B-50 is dephosphorylated not only by the active PP-1-like enzyme, but also by a latent form following treatment of SPM with 0.2 mM cobalt/20 / OH 43699. These results quantitatively support the hypothesis that B-50 is dephosphorylated by a latent form of trypsin in an inhibitor [1 2.6 43] is dephosphorylated in rat cortical synaptic plasma membranes from rat cerebral cortex were used to examine whether AA affects GAP-43 phosphorylation and if it interacts with changes in Ca²⁺ levels. Low concentrations of AA (50 μM) stimulated GAP-43 phosphorylation and that were potentiated by Ca³⁺ (10⁴ M). High AA concentrations (500 μM) decreased GAP-43 phosphorylation to less than that exhibited in the absence of the fatty acid. This bimodal effect was mimicked by another cis-unsaturated fatty acid (18:1c), but not by the trans stereoisomer (18:1t), nor by the saturated isomer of AA (20:0). These results demonstrate that the phosphorylation of GAP-43 is regulated synergistically by free AA and Ca²⁺. This presynaptic regulation may be important in modifying mature synapses and may also play a role in the initial development of neuronal circuitry. Support: NIH NS 25830, EY 05690, MH 14275.

460.6 HISTAMINE AND NICOTINE, VIA DISTINCT MECHANISMS, STIMULATE PARALLEL INCREASES IN SYNAPSIN II PHOSPHORYLATION AND NOREPINEPHRINE RELEASE. S.A. Farmer, R.A. Fittipaldi and M.D. Berridge, Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Protein phosphorylation is widely recognized as the primary molecular mechanism for regulation of protein function, and a family of phosphoproteins known as the synapsins are thought to be involved in neuronal storage. Indeed, a recent report demonstrated that synapsin II may play a role in production of synaptic vesicles and in synapse formation. (Han et al., Neuron, 49, 697-706, 1988). We have also demonstrated that synapsin II phosphorylation and its relationship to norepinephrine (NE) release from bovine adrenal medial chromaffin cells (BACC). This is a complex phenomenon modulated by cholinergic and non-cholinergic transmitters. We have now shown that these two transmitters increase PO₄ incorporation into synapsin II. We report here that maximal doses of nicotine (100 μM) and histamine (100 μM), when combined, produce additive increases in both NE release and synapsin II phosphorylation. These data suggest different mechanisms of activation. We also find that nicotine-stimulated release is abolished in the absence of extracellular calcium, whereas histamine-stimulated release remains significantly elevated in calcium-free media. Nicotine is known to act through nicotinic receptors, stimulating a depolarization-dependent influx of extracellular calcium. Histamine acts through H1-receptors, stimulating a release of intracellular calcium via IP₃ production. Thus, calcium arising from two separate pools can stimulate additive increases in NE release and synapsin II phosphorylation in BACC. In addition, these data provide evidence for the hypothesis that synapsin II phosphorylation plays a role in NE release from these cells. Supported by PHS grant DK40496 to MDB.

460.7 OPTICAL MEASUREMENT OF SYNAPTIC VESICLE RECYCLING. W.J. Betz and G.S. Bewick*, Dept. of Physiology, Univ. of Colorado Med. Sch., Denver, CO 80262.

We have studied the pattern of dye loading and unloading in frog cutaneous pectoral motor nerve terminals using FM-4-6, a derivative of BHA (Molecular Probes, Inc.). FM-4-6 stains synaptic vesicle membranes, producing bright spots along the length of nerve terminals, each consisting of a clump of stained vesicles. We tested the hypothesis that recycled vesicles move randomly within a vesicle cluster. Results from experiments using SPM were further displayed by using exogenous phosphorylase a and histone H1 as substrates. OA-induced inhibition of B-50 dephosphorylation was also demonstrated in rat cortical synaptosomes by use of an immunophenotypic assay with affinity-purified anti-B-50 IgG. These results provide further evidence that SPM-bound PP-1 and PP-2A-like enzymes that share considerable similarities with their cytosolic counterparts may act as physiologically important phosphatases for B-50. (Supported by the Ohio Department of Aging and NH (NS23598)).

460.8 SYNAPTIC VESICLE-ASSOCIATED CaM KINASE II BINDS THE C-TERMINAL REGION OF SYNAPSIN I. E. Benfenati, F. Valerio*, A.J. Cerruti and P. Greengard, Inst. of Human Physiology, University of Milan, Italy; The Rockefeller University, New York.

Synapsin I is a major neuron-specific phosphoprotein which associates with small synaptic vesicles at multiple sites in a phosphorylation-dependent fashion. In an attempt to identify vesicle proteins which interact with the C-terminal "tail" region of synapsin I, purified NTCTCbleaved tail fragment was conjugated to a heterobifunctional cleavable reagent. The SAD-3 of synapsins is the 14-17 kDa tail fragment of synapsin I. If synapsin I is present on the vesicles in close association with CaM kinase II, we would expect to detect the C-terminal CaM kinase II binding domain on synapsin I. We tested whether the binding of the tail fragment is competed by peptides corresponding to the sequences surrounding phosphorylation sites 2 and 3 of synapsin II bound by CaM kinase II inhibitinhibitory peptides based on the sequence of the bovine tail fragment of synapsin II. We found that, under basal conditions, the CaM kinase II peptides (273-302)AA²² and (283-302)AA²² were both effective in displacing the fragment from synaptic vesicles (IC₅₀ = 10 and 35 μM, respectively), the site 3 peptide was less potent (IC₅₀ = 450 μM) and the site 2 peptide was ineffective. The results indicate that CaM kinase II associated with synaptic vesicles is involved in binding the tail region of synapsin II. If synapsin II is present in the vesicles in close association with CaM kinase II, its phosphorylation, stimulated by an increased intracellular Ca²⁺ concentration, could be an extremely fast process, conceivably involved in mediating exocytosis.
460.9 LABELING OF SYNAPTIC VESICLES IN LIVING NEURONS WITH PROPERTIES SIMILAR TO THE A-CURRENT AND DELAYED RECTIFIER CURRENT WERE SEEN. DOPEINE IS A MAJOR NEUROTRANSMITTER IN THE STRIATUM, WE EXAMINED THE POSSIBILITY.

460.10 LOW pH PREVENTS SYNAPTIC VESICLE RECYCLING IN SNAKE MOTOR NERVE TERMINALS. I.C. Marshall*, C. Price*, K. Pemberston* and S.M. Parsons, Department of Pharmacology and Pharmacodynamics, University of Strathclyde, Glasgow, U.K. and Department of Chemistry, UCSB, Santa Barbara, CA.

We have previously shown that prolonged motor nerve stimulation induces the appearance of two populations of miniature end-plate currents (MEPCs) at the snake neuromuscular junction. Similar MEPCs have been attributed to the release of recycling quanta. It is not clear why these recycling quanta are smaller than the normally released pre-formed quanta. Two possible explanations are: (i) that the cytoplasmic supply of acetylcholine (ACH) for these recycling quanta is limited or (ii) that the synaptic vesicle proton gradient, required for the translocation of ACH into the synaptic cleft, is not sufficiently high to support these recycling quanta.

Supported by the Wellcome Trust and the Medical Research Council.

CALCIUM CHANNELS: MULTIPLE TYPES

461.1 PREFERENTIAL RECRUITMENT OF THE DIHYDROPYRIDINE-SENSITIVE COMPONENT OF ACTION-POTENTIAL-INDUCED CA++ INFUX. T. M. Peer and S. A. Thayer, Department of Pharmacology, University of Minnesota Medical School, Minneapolis MN, 55455.

Intracellular free calcium concentration (Ca++) transients were elicited by electrical field stimulation and measured by indo-1 based microfluorometry in single rat hippocampal neurons grown in primary culture. Ca++ currents were mediated by action potentials as indicated by a response threshold sharply dependent on stimulus amplitude, and complete block by tetrodotoxin (1 μM). Ca++ transients elicited by 10 pulses at 10 Hz (peak 540 ± 42 mM from basal 159 ± 16 mM, n=7) were only slightly sensitive to inhibition (1 ± 4%) by intraneurite (NT, 1 μM). Application of tetraciumaene (TEA, 2 mM) increased the amplitude of the response by 290 ± 60%. The TEA-recruited response was blocked by NIT (94 ± 9%, n=4). Stimulation at 50 Hz for 30 pulses increased the amplitude of the Ca++ transients by 192 ± 21% relative to that observed at 2 Hz. NIT did not effect the low-frequency response, but showed a marked inhibition of the high-frequency-recruited response (50 ± 7%, n=3). Previous results have shown that TEA (1) and high-frequency electrical stimulation (2) slow action potential repolarization by decreasing K+ current. Preferential recruitment of dihydroypridylene-sensitive Ca++ influx by increased action potential duration may depend on the slow activation and inactivation kinetics of the dihydroypridylene-sensitive, voltage-activated Ca++ channel.


461.2 POTENTIATION OF THE LOW-VOLTAGE-ACTIVATED (LVA) CA++ CURRENT BY ALBUMIN. S. Huck, M. Freissmuth*, S. Boehm* and S. Hess*. Departments of Neuropharmacology and Pharmacology and Pharmacodynamics, University of Vienna, A-1090 Vienna, Austria.

Bovine serum albumin (BSA) has been reported to enhance LVA Ca and Na currents in chick and rat dorsal root ganglion neurons. In the present study, we have purified BSA with FPLC techniques, confirmed the purity of the molecule enzymatically with trypsin and tested the fragaments for biological activity on the LVA Ca current. Patch clamp recordings (whole cell configuration) were performed on acutely dissociated El3 chick dorsal root ganglion (DRG) neurons with 20mM Ca in the external solution. While not affecting HVA Ca currents, BSA enhanced peak amplitudes of the LVA Ca component with little effect on activation or inactivation kinetics. Half maximal enhancement occurred at approximately 1mg/ml BSA.

Upon chromatography on a MonoQ anion exchange column, the biochemical activity co-migrated with the 67kd protein. Enzymatic cleavage with trypsin yielded prevalent fragments with apparent molecular weights between 20 and 50kdA. On the MonoQ column, the trypsinized products eluted as three major protein peaks. Based on its protein content, the first peak (primarily containing 7, 10 and 26kdA components) was about 15 times as active as the peaks two and three (containing fragment around 40kdA and albumin, respectively).

Our experiments indicate that the LVA potentiating activity is an integral part of albumin and that molecular weight fragments retain this biological effect.

Supported by the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung.

461.3 HIGH AND LOW-_THRESHOLD CALCIUM CURRENTS IN ACUTELY ISOLATED STRIATAL NEURONS. K. Hoehn, T.W.J. Watson and B.A. MacVicar, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N1N1.

The neostriatum is intimately involved in the control of locomotion. Ca++ channels are important in determining the firing patterns of a variety of neurons. The nature and possible modulation of voltage-dependent Ca++ channels has not to date been described in mature striatal neurons. The ionic currents in acutely isolated neurons from rat (21 to 28 day postnatal) striatum were investigated using the patch clamp technique. Cell capacitance of less than 10pF and a population of large neurons with greater than 12 pF capacitance was found to be identical even when the 1 hr incubation with p65-Abs was followed by 2-5 days incubation without antibodies. If during this time synapses were formed, p65-Abs became clustered at sites of contact with SVs. These observations suggest that binding of p65-Abs to the luminal domain of p65 does not significantly affect the localization and traffic of the protein. Our results suggest that p65-Abs may be a powerful marker for SVs and nerve terminals in living neurons.


Numerous studies using intracellular voltage recording have suggested that dopamine (DA) neurons in the rat substantia nigra zona compacta (SNc) possess at least 2 distinct calcium currents (ICa). To further examine the properties of ICa in these cells, whole-cell recordings were obtained from DA neurons in horizontal slices of rat midbrain. Such cells are identified by the presence of tyrosine-hydroxylase, could be divided morphologically into a population of small neurons with a whole cell capacitance of less than 10pF and a population of large neurons with greater than 12 pF capacitance. A TTX-Sensitive Na+ current as well as K+ currents with properties similar to the A-current and delayed rectifier current were seen.

Supported by the Wellcome Trust and the Medical Research Council.

462. ANTIBODIES TO THE LUMENAL DOMAIN OF SYNAPTOTAGMIN/P65 LABELING OF SYNAPTIC VESICLES IN LIVING NEURONS WITH p65-Abs and for synaptic vesicle antigens was found to be identical even when the lhr incubation with p65-Abs was followed by 2-5 days incubation without antibodies. If during this time synapses were formed, p65-Abs became clustered at sites of contact with SVs. These observations suggest that binding of p65-Abs to the luminal domain of p65 does not significantly affect the localization and traffic of the protein. Our results suggest that p65-Abs may be a powerful marker for SVs and nerve terminals in living neurons.
461.5

"N"- and L-LIKE CALCIUM CURRENTS IN LUNG CANCER CELLS ARE BLOCKED BY LAMBERT-EATON IgG.  S.C. Hulauer, S.D. Meretsky, A.D. Grinstein and V.A. Lennon.  
Juni Lewis Neuromuscular Res. Ctr., UCLA School of Medicine, Los Angeles, CA 90024 and Mayo Clinic, Rochester, MN.  
Lambert-Eaton syndrome (LES), an autoantibody-mediated disorder of presynaptic calcium channels at the neuromuscular junction (NMJ), is often associated with small cell lung cancer (SCLC). We are therefore studying the current in human SCLC cell lines that express both "N"- and L-like calcium currents in an attempt to characterize the type(s) of calcium channels that may exist at the NMJ. Whole-cell and cell-attached patch clamp recordings from the NCI-H345 cell line reveal two types of high-threshold calcium current (carried by barium). The "L"-like current is non-inactivating; 50%f state-inactivated at -10 mV, sensitive to dihydropyridines, and with a single channel conductance of about 21 pS. The "N"-like current is more sensitive to channel inactivation: during a 100 ms pulse, is 50% state-inactivated at -50 mV, insensitive to dihydropyridines, and has a single channel conductance of about 16 pS. This is the first demonstration of an "N"-like channel in a non-neuronal cell. Both currents are significantly reduced following 72-hour incubation in LES IgG and unaffected by IgG from myasthenia gravis patients. Supported by grants from the MDA, NIH (CA57343, NS06252) and NSF (NS8919041).

461.6

Miles Inst. Preclin. Pharm., West Haven, CT 06514.  
Calcium channel currents have been studied using whole-cell and cell-attached patch recording in a cell line derived from N1E-115 (passages 20-40). After 2-5 days in culture these cells have only low threshold current (n=30). Culturing with DMSO (2-10 µM) in an attempt to differentiate these cells failed to enhance neurite formation or induce high threshold currents. Elicitable currents activated near -55 mV, peaked near -15 mV and reversed positively to +40 mV (20 Ca++)/120 Cs+). Substitution of barium for calcium and calcium-free carrier buffer did not enhance inward current or alter the voltage dependence of channel inactivation. The midpoint of the steady-state availability curve was -58.0 mV (n=5) with no elicitable current from depolarized holding potentials (Vh = -30 mV) even with strongly depolarizing test potentials (Vt = 20 mV). Tail currents with slow T-type kinetics (Vt=-65; r=6 msec) were well fit by single exponentials. Cell-attached patch recording (n=6) characterized the underlying conductance as a 7.5 pS channel (110 Ba++) . This preparation provides an opportunity to study neuronal T-type calcium channels in isolation from N- or L-type.

461.7

PHARMACOLOGY OF T-TYPE CALCIUM CHANNELS IN A NEURONAL CELL LINE.  F.E. TanPieneco and R.T. McCarthy.  
Miles Inst. Preclin. Pharm., West Haven, CT 06514.  
The pharmacology of T-type calcium channel current was studied in a neuronal cell line derived from N1E-115 that expressed only T-type currents. These low threshold currents (activation at -55 mV) could be inhibited by nickel (150 µM) and completely inhibited by cadmium (500 µM). Omega-conotoxin (5 and 10 µM) had no effect on currents. Neither whole-cell peak current nor tail current were enhanced by BAY K 8644 and, instead, appeared to be slightly inhibited by this drug. 

The agonist enantiomer of BAY K 8644 (BAY K 5417 (100 µM)) had no effect on this low threshold current. Studies with cell-attached patch recording (120 Ba++) in the presence of calcium channel agonist failed to demonstrate large conductance openings or a switch in the mode of gating of the 7.5 pS conductance. Activity of the 7.5 pS channel was retained in excised patches. This calcium channel current shares the pharmacological characterization of T-type channels, providing an opportunity to study the modulation of T-type channel current isolated from other neuronal calcium channel types.

461.8

DIPHENYL BUTYLPiperidine (DPBP) ANTISPASMODICS PREFERENTIALLY BLOCK T-TYPE CALCIUM CURRENTS IN NEURAL CREST DERIVED THYROID C. CELLS.  L.J. Enyeart*, B.A. Blagia, and B. Millar.  
Depts. of Pharmacology and Physiology, The Ohio State University, Columbus, OH 43210-1239.  
The inhibition of Ca2+ channels by the DPBP penfluridol was studied with whole cell patch clamp in thyroid C-cell lines. Three separate components of Ca2+ current, corresponding to T, L, and N channels, were identified in rat MRTC 6-23 (clone 6) cells. The low voltage-activated, rapidly inactivating T current was potently blocked by penfluridol. At a concentration of 500 nM, penfluridol inhibited 68.0 ± 2.3% (n=28) of T current. The slowly inactivating component of Ca2+ current was blocked by penfluridol in the human TT C-cell line. Block of T current by penfluridol was use-dependent and promoted by channel activation. T-type Ca2+ channels were also effectively blocked by penfluridol in the human TT C-cell line. Among organic Ca2+ antagonists, DPBPs are distinctive in their ability to preferentially block T-type Ca2+ channels at low concentrations. DPBPs will be useful in determining the function of this Ca2+ channel subtype in excitable cells.

461.9

LOW THRESHOLD CALCIUM CURRENT OF RAT LATERODORSAL TECTINAL NEURONS STUDIED WITH WHOLE-CELL PATCH CLAMP IN SLICES.  A. Kasneci, B. Reuter.  
Kneser Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.  
As an integral part of the ascending reticular activating system, the interstitial nuralis (LDT) nucleus plays an important role in the regulation of damicotonic function. Intracellular recordings have shown that there exist rat that express, by different classes of neurons, the LDT soma. We have used the whole-cell patch clamp technique as applied to the in vitro slice preparation to study the characteristics of the transient calcium current, I1, evoked by depolarizing voltage steps to -50 mV from a holding potential of -45 mV. Activation of I1 showed extremely slow voltage dependence in the voltage range from -55 mV to -45 mV. The time-to-peak latency became shorter at more depolarized voltages. The decay phase of the current followed a single exponential time course, with an average tau of 70 msec. Activity of the 7.5 pS channel was retained in excised patches. This calcium channel current shares the pharmacological characterization of T-type channels, providing an opportunity to study the modulation of T-type channel current isolated from other neuronal calcium channel types.

461.10

T-TYPE CA++ CURRENT AND ITS FUNCTION IN TRIGGERING CA++ INFLUX IN EMBRYONIC XENOPUS SPINAL NEURONS.  Xiaoxun Gu and Nicholas G. Spitzer.  
Department of Biology & Center for Molecular Genetics, UCSD, La Jolla, CA 92039.  
Previous studies have shown that spontaneous Ca++ influx during a critical period plays an important role in development of embryonic Xenopus spinal neurons in culture. We have used the whole cell voltage clamp technique to study low voltage activated (LVA) T-type Ca++ current and its role in regulating Ca++ influx during neuronal development. Blocking high voltage activated (HVA) N- and L-type Ca++ currents with intracellular F- and replacing or removing extracellular Ca++ showed that LVA T-type Ca++ current is present in these cultured neurons. This T current is a transient inward current and can be activated at membrane potentials between -60 and -45 mV. It has the lowest threshold among the voltage activated depolarizing currents recorded in young neurons, and reaches its peak at membrane potentials around -35 mV with an amplitude of 50 pA. The rates of both activation and inactivation are voltage dependent. With steps to -35 mV, the time to half maximal activation and the time constant of inactivation are 3.6±0.2 ms and 17.5±1.8 ms, respectively. The T current is present in both young and mature neurons with no apparent change in its properties. N+ has been shown to block increases in intracellular Ca++ triggered by Ca++ influx. In the present study, we found that N+ can be used to selectively block T current with only a minor effect on HVA currents. LVA T current may be involved in depolarizing cells and triggering HVA Ca++ currents, thus inducing Ca++ influx in cultured neurons at early stages in their differentiation. Supported by NIH NS15918 to NGS.

Skeletal muscle displays two types of inward Ca" currents: a slow, dihydropyridine sensitive current and a transient current which is less sensitive to dihydropyridines. The purpose of the present study was to determine if the developmental appearance of these two currents was influenced by the presence of the nerve. Muscle cells were removed from embryos of the frog Xenopus laevis at early stages (12-14) of development, 4-6 hr prior to innervation, and plated in culture (nerve-free, NF). The cells were then compared to those in muscle cells derived from older embryos (stage 19-21) and grown in the presence of neural tube cells (nerve-enriched, NE). Ca" currents were examined using the whole cell recording technique in the absence of Na" or K" (extracellular solution, in mM: 127.5 tetraethylammonium, 20 BaCl2, 1.2 MgCl2, 10 Hepes; electrode solution, in mM: 140 CsCl, 5 MgCl2, 1 BaCl2, 10 Hepes; both solutions were pH 7.4). There was no difference between the NE and NF cultures in the number of cells with a transient or slow current, the developmental appearance of the two currents, or the current density. The two currents however, differ from each other in their developmental time course. By the first day in culture about 60% of the cells have a slow current compared to 11% with a transient current. By day 2 all cells had a slow current whereas by day 3 only 50% of the cells had a transient current. The results of the present study indicate that although the slow and transient Ca" currents differ from each other in a number of ways the developmental appearance of these currents is not influenced by the presence or absence of the nerve.

461.12 PROPERTIES OF SINGLE VOLTAGE-GATED CALCIUM CHANNELS IN DISSOCIATED MEDIAL SEPTUM/DIAGONAL BAND (MS/DB) NEURONS. N.H. McCleshy and M.J. Carstens. Department of Pharmacology, Center for Drug and Toxicol. and Medical Physiology. College of Medicine, Texas A&M University, College Station, TX 77843.

We have previously shown that both low voltage activated (LVA) and high-voltage activated (HVA) calcium currents in MS/DB neurons using the whole-cell patch-clamp technique (Neurosci. Abstr. 16: 1990). In the present study identified the properties of a single calcium channel contributing to these macroscopic currents. Calcium channels were recorded in MS/DB neurons acutely dissociated from adult guinea pigs (170-700 gms) using the cell-attached recording configuration. Barium (100-110 mM) inside the pipette was used as the charge carrier and isotonic K-ascorbate (140 mM) was used to zero the cell's resting potential. From a holding potential of -80 mV, two channel types were recorded most often; a first a low conductance channel (6-7 pS) that was activated near -40 mV, and second, a large conductance channel (24-26 pS) activated near -20 mV. These latter channels were recorded in the presence of Bay K 8644 (1-5 μM). A third channel type (12-16 pS) was recorded less frequently and was activated near 0 mV. These three channel types differed in their voltage-dependence, open-probability and sensitivity to Bay K 8644 and may resemble T, L and N type channels recorded in hippocampal and ganglion cells. (Supported by AO7805, HL-38104, AR-8612.)

Venoms from the fish hunting small species, Conus geographicus and C. magus, possess two omega-conotoxins GVIA (ω-GVIA). Preliminary structure-activity relationships (SAR) have demonstrated that this highly active venom, containing three intracellular disulfide bonds, interacts with a subpopulation of mammalian neuronal Ca++ channels. To further evaluate the SAR of ω-GVIA, biological assays were performed on two Ca++-selective fluorescent dyes (i.e., ω-GVIA binding to hippocampal membranes; Ca++ influx in chick sympathetic) were obtained following either chemical modification or primary a.a. alteration of ω-GVIA.

Acetylation of primary amino moieties of Cys-1, Lys-2 and Lys-24, under non-saturating conditions, generated 7 (1 mono-, 3 di- and 1 tri-acetylated) distinct peptides. Positional assignment of acetylation was accomplished using a combination of tryptic digestion, RP-HPLC and MS. Biological assays indicate that successive acetyl additions lead to a loss of activity. From binding analyses, the N-terminus amino of Cys-1 is more significant than the ω-amino moieties of either Lys-2 or Lys-24. Selective modifications of the primary structure of ω-GVIA will be explored to evaluate the physiological relevance of other basic moieties and of secondary structural features as a result of disulfide bridging.


Previous investigations in this laboratory have demonstrated that mouse cortical astrocytes express a physiologically functional ω-conotoxin-sensitive binding site. In the current study, results suggested that astrocytes possess a binding site specific to the VSCC type of ω-CgTx. The attempts to inhibit ω-CgTx in binding to the astrocytes with unlabeled α-CgTx were unsuccessful. These results support the idea that the production of a specific binding site for a conotoxin is a general property of certain VSCC types. The evidence suggests that the production of a specific binding site for a conotoxin may be a general property of certain VSCC types.


Synthetic ω-conopeptides such as SNX-111 from Conus magus (see abstract by Miljanich, et al.) specifically block neuronal calcium channels. An SNX-111 receptor and putative calcium channel has been localized to the presynaptic membrane of nerve terminals of the electric organ of the electric ray, Narcine brasiliensis. The detergent solubilized receptor was purified 2,000-fold to near homogeneity using wheat germ agglutinin chromatography and SNX-111 affinity chromatography. In solution, the native receptor appears to be a multi-subunit complex (Mr=1,500,000-2,000,000) as shown by SDS-PAGE under nonreducing conditions. The molecular weight of the receptor shifted in two steps upon exposure to reducing agent. A single band of Mr=350,000 was observed with 0.1M DTT and a doublet of Mr=200,000 and Mr=180,000 was observed with 25mM DTT. Cross-linking experiments with intact synaptosomes provided further evidence that the purified protein is the presynaptic conopeptide receptor.

That is, 125I-SNX-111 specifically labeled a band of Mr>1,000,000 under nonreducing conditions and a single band of approximately 210,000 under reducing conditions. Thus, it is likely that one of the affinity purified polypeptides contains the conopeptide binding domain and that it is disulfide-linked to the other polypeptide to form a heterodimer. Moreover, several heterodimers are linked through disulfide bonds to form a multi-domain complex.


Synthetic ω-conopeptides such as SNX-111 from Conus magus and SNX-183 from Conus textile appear to define subtypes of brain voltage-sensitive calcium channels (VSCC's). In rat brain synaptic-somal membranes, 111 was more potent than 183 in displacing binding of [3H]nimodipine and [3H]PN200-110 and 2,4M respectively whereas 111 was more potent than 183 at displacing [3H]nimodipine or [3H]PN200-110. The present investigation was initiated in an attempt to ascertain whether nimodipine and ω-CgTx do interact with the same binding site. Results obtained thus far have demonstrated that [125]ω-conotoxin binding increases pari passu with [3H]nimodipine binding through the fourth culture week. After the fourth week in culture, [3H]nimodipine binding decreases with culture age; whereas, [125]ω-conotoxin binding plateaus. These results suggest that the ligands for the N- and L-type VSCC's are not interacting with the same binding site. Furthermore, physiological studies conducted with the Ca++-sensitive fluorescent probe Indo-1 have demonstrated that ω-conotoxin completely blocks KCI (5mM) induced [Ca++2]i transients, whereas nimodipine only stimulates KCn-induced [Ca++2]i transients. The expression of distinctly different VSCC types on astrocytes may provide important pharmacological targets for drugs useful in the treatment of seizure disorders and stroke. Supported by NIH grant 2-R01-NS2200 from the National (NIH).


Synthetic ω-conopeptides (see abstract by Miljanich, et al.) block the potassium induced release of radioactively-labeled (3H) noradrenaline (NE) or dopamine (DA) from rat hippocampal slices. SNX-111 blocked NE release partially (50%) with an IC50 of 1 nm whereas SNX-183 inhibited K+ induced release completely with an IC50 of 400 nM. SNX-111 was much more effective in blocking DA release (IC50 160 nM) than was SNX-111 (IC50 > 1000 nM).

The results of experiments confirm that the conopeptides block neurotransmitter release in a more physiological setting. EPSP field potentials elicited by electrical stimulation in rat hippocampal slices in vitro were reversibly blocked by a variety of Neurex conopeptides and again were more completely blocked by SNX-183 (~100%) than SNX-111 (~50%). The compounds also blocked neurotransmitter release in vivo in the rat. Extracellular concentrations of glutamate and glutamine revealed no significant increase in baseline or microdialysis during potassium infusion through the probe were inhibited in the hippocampus and thalamus by about 50% by SNX-111 as compared to the levels of the neurotransmitter amino acid sera.

These techniques provide functional evidence for the presence of multiple subtypes of voltage gated calcium channels and thus extend the binding data presented in the abstract by Miljanich, et al.
**4.61.11**

**METABOLISM OF VOLTAGE-OPERATED CALCIUM CHANNELS IN HUMAN NEUROBLASTOMA CELLS.** E. Sher*, M. Passafaro*, F. Spire* and F. Clément*. CNR CRI, G. 

**4.62.11**

**DOSE RESPONSE RELATION FOR SYNTHETIC FTX BLOCK OF VOLTAGE-DEPENDENT CALCIUM CURRENTS IN DISASSOCIATED PURKINJE CELLS.** M. Sugimoto, R. Chekry and R. Ungar. Dept. of Physiology and Biophysics, NYU Medical Center, New York, N.Y. 10016.

**4.62.12**

**DISSOCIATED CEREBRAL PURKINJE CELLS FROM GUINEA PIG WERE STUDIED USING WHOLE CELL RECORDING TECHNIQUES TO EXAMINE THE EFFECTS OF A SYNTHETIC NMDA RECEPTOR AGONIST, (+) SDZ 202-791.** (-) SDZ 202-791, antagonized the PMA-induced enhancement in K^+^-stimulated release of [3H]5-HT due to Bay K 8644 (0.2 μM) in the presence of 15 mM K^+^ increased release of [3H]5-HT 30-40%. In competition studies, unlabeled trypsin dispensed to compete with a K value of 7 nM. Binding was found to be dependent on the concentration of free Ca^2+^ with an EC_50 value of 139 μM. Binding was reduced by 25% in the presence of 5 mM Mg^2+^ and was restored with the addition of 10 mM caffeine. Caffeine alone did not significantly alter the levels of binding. The distribution of [H]ryanodine binding sites in human brain was heterogeneous with high levels observed in putamen and caudate nucleus, intermediate levels in cortex, hippocampus and globus pallidus, and low levels in cerebellum. The high levels of binding seen in basal ganglia suggest that ryanodine receptors may be particularly important here in the regulation of intracellular calcium and may be relevant to such disorders as neuroleptic malignant syndrome, Huntington's disease, and Parkinson's disease.

**4.62.15**


**4.62.16**


**4.64.3**

**EFFECTS OF (+) AND (-) ENANTIIOMERS OF SDZ 202-791 ON 15mM K^-STIMULATED [H]RYANODINE RELEASE IN RAT SPINAL CORD SYMPTOMOSOMES.** D. Gandhi and D.J. Jones. Dept. of Pharmacology and Toxicology, Univ. Texas Health Sci. Ctr., San Antonio, TX 78284-7838.

**4.64.6**

postsynaptic action of this compound leading to facilitation of partly due to its anticholinergic properties. The enhancement of In neostigmine solution (maximal response) whereas it was antagonized by it was noted. The EPSP-enhancing action of THA (without changing the maximum response) whereas it was antagonized by 10 μM THA. In neostigmine increases responses to low doses of THA but depressed those to large ones. No change in responses to 100 μM carbafos was found. The EPSP-enhancing action of Ach was potentiated by 1 μM THA (without changing the maximum response). No change in responses to 0.5 mM gabazine was seen. These data suggest that THA possesses cholinomimetic activity partly due to its anticholinergic properties. The enhancement of responses to exogenous glutamate by THA indicates an additional postsynaptic action of this compound leading to facilitation of excitatory synaptic transmission.

463.4

5-HT and muscarinic hyperpolarize a subpopulation of neurons in rat nucleus raphe magnus in vitro. K. Kanamori*, S. Harada*, M. Kamitani*. Institute, Oregon Health Sciences University, Portland OR 97201.

Both 5-HT and Ach have been implicated in the immunohistochemical production of nuclear raphé magnus (NRM). Intracellular recordings were made from NRM cells in a slice preparation. 5-HT and 5-HT1 receptor agonists hyperpolarized most NRM neurons. The hyperpolarization (Vmax = 12 mV) was blocked by ketanserin (1 μM), but not dopamine receptor antagonists, suggesting that a 5-HT1 receptor mediated the response. In a proportion of the neurons that were hyperpolarized by 5-HT, muscarinic neurons also caused a dose-dependent hyperpolarization (Vmax = 10 mV). The dose response curve for muscarine was shifted to the right by pertussis (300 nM - 3 μM) and methocholine (90 - 200 μM). Schild regression analysis indicated that a non-M1 (possibly M2) muscarinic receptor is probably involved. Both 5-HT and muscarine caused an outward current with similar voltage dependence, showing inward rectification that was abolished by 4-AP. The maximal potential was about - 90 mV in normal potassium and shifted to less negative potentials when extracellular K+ was increased. In addition, the currents induced by 5-HT and muscarine were not additive. The results suggest that both 5-HT and muscarine inhibit a population of the NRM neurons by increasing the inwardly rectifying K+ conductance, an effect that may be involved in the modulation of opioid analgesia in NRM. Supported by NIH grants DA04523 and MH45003.

463.5


In lower vertebrates neurotransmission between the optic nerve and relay neurones is probably glutamatergic and upregulated by nicotinic stimulation. The action of the cholinergic agent THA, studied mainly with electrophysiological recordings, had results similar to those observed in the thalamus. THA (0.1-1 μM) increased by about 50% the amplitude of population EPSPs elicited by optic nerve stimulation but it failed to do so in intracellular recordings of 100 μM neostigmine. No change in the afferent volley spikes was noted. The EPSP-enhancing action of Ach was potentiated by 1 μM THA (without changing the maximum response) whereas it was antagonized by 10 μM THA. In neostigmine, THA increases responses to low doses of Ach but depressed those to large ones. No change in responses to 100 μM carbafos was found. The EPSP-enhancing action of THA was potentiated by 1 μM THA (without changing the maximum response) whereas it was antagonized by 10 μM THA. In neostigmine, THA increases responses to low doses of glutamate was augmented by 10 μM THA, while no change in responses to 0.5 mM GABA was seen. These data suggest that THA possesses cholinomimetic activity partly due to its anticholinergic properties. The enhancement of responses to exogenous glutamate by THA indicates an additional postsynaptic action of this compound leading to facilitation of excitatory synaptic transmission.
463.7 DUAL PROJECTIONS OF SINGLE CHOLINERGIC AND AMINERGIC BRAINSTEM NEURONS TO THE BASAL FOREBRAIN AND THALAMUS IN THE RAT. Bruno J. Looser and Khare Sembhi. Departments of Psychology and Anatomy, Dalhousie University, Halifax, N.S., Canada B3K 4B7.

Recent evidence indicates that during waking cholinergic basal forebrain neurons are strongly activated, whereas in the thalamus spindle activity is suppressed and sensory transmission facilitated. Both of these subcortical structures receive projections from the basal cholinergic and amine neurons, and these neurons are known to be active during waking. However, it remained unclear whether single transmitter-specific brainstem neurons contributed to both of these ascending projections. In the present study we examined this possibility of dual projections by spike-tracing into the thalamus and basal forebrain, followed by immunohistochemistry with antibodies to cholinergic acetyltransferase, tyrosine hydroxylase, serotonin, and histidine decarboxylase.

Cholinergic neurons projecting to both the basal forebrain and thalamus were found in the pedunculopontine and laterodorsal tegmental nucleus, and noradrenergic dually projecting neurons were seen in the locus coeruleus. However, few serotonergic neurons in the brainstem appeared to have the dual projection. In conclusion, subpopulations of cholinergic and amine neurons in the brainstem have branching axons that innervate both the basal forebrain and the thalamus. Through these dual projections, these single transmitter-specific neurons can concurrently modulate the activity of both subcortical structures during cortical arousal. Supported by the MRC, Alzheimer Society, and Scottish Rite Charitable Foundation of Canada.

463.8 CORTICAL CHOLINERGIC FIBERS IN THE HUMAN BRAIN: AN IMMUNOCHEMICAL STUDY OF THE HIPPOCAMPAL MAMMALIAN FORMATION. C. Liu, S. Kastner. Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637, USA.

The cholinergic innervation of the hippocampal formation is thought to play an important role in regulating memory consolidation. We investigated the organizational aspects of the cholinergic innervation of the human hippocampal formation by using polyclonal antibodies against human placental choline acetyltransferase (ChAT - gift of Dr. L. Hedges) and acetylcholinesterase (AChE - gift of Dr. S. Younkin) in a series of normal individuals. Staining in selected sections was quantified by a modification of the Fontana-Masson method. Fine, varicose ChAT-immunoreactive (ir) fibers were found densely innervating the different hippocampal fields in a laminar pattern that was similar to what was previously reported in other species. In the dentate gyrus, there was dense ChAT-ir innervation of the granular, and the infra- and supragranular layers. Dense ChAT-ir innervation was seen in the CA3 field and mainly in the infrapyramidal layer of CA1, but little innervation was seen in CA2. A much larger number of fine smooth AChE-ir fibers were seen in the hippocampal formation providing a more extensive and somewhat different pattern of innervation than was revealed by the CHAT staining. For example, the CA2 field was the most densely innervated in AChE-stained material. These observations indicate that cholinergic innervation plays a major role in the function of the human hippocampus, but that the AChE-stained fiber distribution does not provide an accurate estimate of the cholinergic innervation.

463.9 PROJECTIONS OF THE BASAL FOREBRAIN TO VISUAL CORTICAL AREAS IN THE CAT. Christopher C. Nabor* and Kenneth E. Kratz, Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Cells of the basal forebrain provide cholinergic input to the entire neocortex. In the present study retrograde tracer techniques (rhodamine labelled latex microspheres and WGA-HRP) combined with ChAT immunocytochemistry were used to examine the topography of the forebrain projections to functionally defined visual cortical areas in the cat. Our results indicate that primary visual cortex (area 17) is heavily innervated by the nuclei of the diagonal band with a less dense innervation from the more lateral and caudal cell groups of the basal forebrain. On the other hand areas 18 and 19 along with the visual association areas of the lateral suprasylvian gyrus and areas 20 and 21 receive a substantial input from the diagonal band nuclei but receive their most dense projection from the substantia innominata, neurons within the putamen and globus pallidus, and neurons scattered within the internal capsule. It appears that the processing of visual information in functionally distinct areas of cortex may be subject to different regulatory inputs from the cell groups of the basal forebrain. The diagonal band nuclei may play an important role in the gating of sensory information into the primary areas of visual cortex while the more lateral cell groups may have a greater influence over higher order processing of visual information.

463.10 PRIMATE NUCLEUS BASALIS NEURONS DEVELOP RESPONSES TO STIMULI ASSOCIATED WITH WATER REWARD. R.T. Richardson Dept. Neurology, Johns Hopkins U Ble, Baltimore, MD 21207.

We have previously reported that a large proportion of neurons of the nucleus basalis in naive monkeys had altered discharge rates following arousing stimuli (water reward, air puff, hypertonic saline). In contrast, far fewer basalis neurons responded to neutral stimuli such as tones and perturbations of the forearm. We now report that many more basalis neurons respond to neutral stimuli that have become associated with a water reward. Conditioning procedures have been completed in one juvenile male rhesus monkey. In the first phase of testing, one of two tones (150 Hz to the left or right applied to the tonewall) was a CS+ which preceded a water reward by 1.5 to 2 s, and the other stimulus was the same modality (CS-) which never preceded a water reward. After several months, one of two tones (500 Hz and 1000 Hz) became the CS+ and the other was the CS-. The CS+ and CSwere preferentially responded to. In the naive monkey, 21% of 141 basalis neurons responded to the somatosensory stimuli, but in the conditioned animal, 72% of 68 neurons responded to tones in the naive animal, compared to 55% of 78 neurons in the conditioned animal. Of these neurons that responded to tones, 42% had larger responses to the CS+, and 5% had larger responses to the CS-. These findings provide further evidence that many basalis neurons are primarily responsive to stimuli that are inherently arousing to the animal (aversive or appetitive stimuli) or that have become arousing through conditioning procedures.

463.11 CORTICAL AND BASAL FOREBRAIN NEURONAL ACTIVITY FOLLOWING PERIPHERAL NERVE TRANSECTION. H.H. Webster and R.W.Dykes. Dept. of Physiology, University of Montreal, Montreal, Canada, H3C 3T7.

Recent reports have shown that acetylcholine (ACh) acts as a neurotransmitter to influence cortical plasticity and structurally changes of the cortex during the recovery period after peripheral nerve transection. Some of these observations have been deduced from a depletions of the transmitter following destruction of cells in the basal forebrain or of the fibers projecting from those cells. However, the mechanisms by which these changes may be playing an important role in the peripheral nerve lesions we recorded spontaneous, extracellular single unit activity from the femoral cortex at AP -1.8 and ML 3.0, sampling continuously from the surface of the cortex to 8.0 ml below the surface where cholinergic cells of the basal forebrain are located. The location of the electrode tract and tip was confirmed in sections stained for Nissl substance. These results show that in the cortex and basal forebrain, in general spontaneous activity is higher and spike discharge are larger than those seen in the peripheral tissue, and support idea that the basal forebrain participates in the reorganization process following peripheral nerve lesions.
463.13


Both disopropylfluorophosphate (DFP) and soman are irreversible inhibitors of cholinesterase (ChE). DFP is used widely to investigate cholinergic function, while soman is a chemical warfare agent. Last year at this meeting presented evidence suggesting that DFP has some noncholinergic effects. Soman was used in this study to validate the previous findings with DFP.

All experiments were done on fully acclimatized adult cats. Following collection of baseline visual evoked responses (VER) and measurement of blood ChE activity, i.e., soman was administered. VERs then were recorded and ChE activity measured periodically.

Preliminary results from eight cats show a preferential loss of low spatial frequency information similar to that following DFP. The VER loss can be reversed, at least partially, with atropine. At similar levels of acetylcholinesterase activity, blood pseudocholinesterase inhibition, ChE inhibition in visual cortex, and VER reduction is less after soman.

Neurochemical changes in tissue from visual cortex also will be discussed.

463.15

DIRECT CYTOTOXIC EFFECT OF THE CHOLINERGIC TOXIN AF64A ON CEREBRAL CAPILLARY ENDOTHELIAL CELLS IN CULTURE. C. Goméz*, C. Wert and C. Estrada. Dept of Physiology, Sch of Medicine, UAM, Madrid.

The ethylcholine mustard aziridinium ion (AF64A)has been reported to selectively destroy cholinergic neurons, and is currently used to provide animal models of cholinergic neurodegeneration. A previous study in the rat retina showed that, in addition to cholinergic neuron damage, ultrastructural alterations were also observed in endothelial cells. The purpose of the present investigation has been to evaluate and compare the direct cytotoxic effect of AF64A on cultured cerebral capillary endothelial cells (CCE), on a choline acetyltransferase-containing neuroblastoma cell line (IMR32), and on cultured cerebrovascular smooth muscle cells (CVSM).

Cells grown in 24 well plates were washed with PBS, and incubated with DME containing different concentrations of AF64A (1-100 μM) for 2 or 24 hours. Control and sham-treated cultures were run in parallel. After the incubation period, media were collected, centrifuged and the supernatant was used for lactate dehydrogenase (LDH) measurements. Cell injury index was calculated as [(A/CA) X 100], where CA and TA represent LDH activity in the media from control, AF64A-treated, and TX100-treated cultures respectively.

Two hour incubation with AF64A did not increase LDH release in CCE or CVSM, however, a slight enhancement in LDH concentration was observed in IMR32 supernatants after exposure to 100 μM AF64A. After 24 hour, morphological alterations and floating cells were observed in CCE and IMR32 cultures. Accordingly, LDH release increased in a concentration-dependent manner in CCE and IMR32 treated with AF64A, but no changes were observed in CVSM cultures. Half of the maximal cell injury was obtained with 30 μM AF64A in CCE, and with 15 μM AF64A in IMR32.

These results show that a) AF64A has a direct toxic effect on cerebral endothelial cells, b) the sensitivity of cultured endothelial cells is close to that found in neuroblastoma cells, and c) such cytotoxic effect is not nonspecific, because it was not observed in cerebrovascular smooth muscle cells.

EXCITATORY AMINO ACIDS: RECEPTORS IV

464.1

REGULATION OF NMDA RECEPTORS BY GLYCINE AT SYNAPSES AND IN OUTSIDE-OUT PATCHES. G. Tong, R. A. J. Lester* and C. E. Jahr. Vollum Institute, Oregon Health Sciences University, Portland OR 97201

Although glycine is a required co-agonist for the activation of NMDA receptors, its modulation of synaptic NMDA function is less clear. The actions of glycine agonists and antagonists on postsynaptic NMDA currents recorded between pairs of hippocampal neurons in culture have been studied. Additionally we have examined these interactions on NMDA receptors in outside-out patches. Synaptic stimulation of NMDA receptors is mimicked by brief application (~ 5 ms) of L-glutamate (200 μM) to outside-out patches, which in the presence of saturating concentrations of glycine (10 μM) produces a long-lasting response. In the absence of glycine and/or the presence of glycine antagonists an NMDA response is barely detectable. Subsequent addition of glycine at various intervals following brief glutamate application to transmitter-liposome-glutamate allows NMDA channels to open and produces a current with a time course and amplitude dependent on the interval. Following synaptic stimulation in the presence of glycine, rapid removal of glycine has little effect on the decay of the NMDA current, indicating that glycine binds with high affinity, whereas removal of a lower affinity ligand, L-alanine, rapidly curtails the synaptic NMDA current. These data are consistent with the requirement for occupation of the glycine site to allow NMDA channels to open.

Supported by McKnight Endowment Fund for Neuroscience and NIH grant NS21419.

464.2

GLUTAMATE OPENS NMDA CHANNELS WITH A HIGH PROBABILITY C. E. Jahr. Vollum Institute, Portland, OR 97201.

NMDA receptor-channels require both glutamate and glycine binding sites to be occupied for channel opening to occur. If free glutamate is present only briefly, as is probable at excitatory synapses, then the agonists could unbind before the channel has the chance to open. Outside-out patches containing many NMDA channels were used to determine the probability of a liganded channel opening. Four ms pulses of a saturating concentration of glutamate (200 μM) were applied to patches in the absence and presence of 20 μM glycine every 10 or 15 s. After stable responses were obtained, one pulse of glutamate was applied in the continued presence of the channel blocker, MK-801 (10 μM), which at 40 μM will block open, but not closed, NMDA channels in about 2-3 ms, on average. These channels will remained blocked for prolonged periods if held at -60 mV. Responses to subsequent glutamate pulses (in the absence of MK-801) resulted in currents about 30% of control. These results indicate that once NMDA channels are fully liganded, the probability that they will open is about 0.7. The currents activated in the presence of MK-801 describe a first-order latency distribution and indicate that of those channels destined to open, over 80% open for the first time within about 30 ms of glutamate binding. If NMDA channels in outside-out patches mimic those at the synapse and spontaneous synaptic release results in receptor saturation, then there must be very few NMDA receptors present adjacent to release sites as spontaneous NMDA minis are very small (Bekkers and Stevens, Nature 341:230, 1989). Supported by the McKnight Endowment Fund for Neuroscience and NIH grant NS21419.
464.3 EFFECTS OF ANRACETAM AND WHEAT-GERM AGGLUTININ ON FAST EXCITATORY SYNAPTIC TRANSMISSION IN CULTURED HIPPOCAMPAL NEURONS. W. G. Bullitt, M. L. Mayer, E. E. Fox, and E. L. Maguire. Section on Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

The rapid time course of desensitization at AMPA/kainate receptors suggests that desensitization is an integral part of the excitation of synaptic currents. 

Receptor Density in Excised Membrane Patches. The kainate agonist domoate (500 μM) increased the amplitude and slowed the decay time constant of EPSCs by 1.8 and > 2-fold, respectively. Variance analysis indicated a postsynaptic action. The kainate- and domoate-evoked currents are both slowed by 2-fold in the presence of 500 μM CNQX or by 5 mM kynurenic acid. During voltage clamp recordings (Vhold = -60 mV) spontaneous, small inward currents were apparent. In the presence of both drugs, Tg = 47 ± 5.9 ms.

Anion effects on EPSCs suggest that desensitization at AMPA/kainate receptors may affect both the amplitude and time course of synaptic currents. The smaller effect of WGA is consistent with its effect on L-glutamate, NMDA, and kainate receptors. These rapid kinetics may be refuted in the fast time course of synaptic currents in the chick retinal ganglion cell.
464.9


Recent studies have shown that polyamines potentiate NMDA-induced currents in neurons injected with rat brain mRNA (McGehee et al., PNAS 87: 9971, 1990), as well as increasing the binding of [3H]glycine and [3H]-MK-801 to the NMDA receptor complex. These observations suggest a physiological role for polyamines in modulating NMDA-evoked responses. To gain further insight into the actions of polyamines on NMDA receptors, we studied the effects of the polyamine spermine on NMDA-induced currents in rat hippocampal neurons. At the whole-cell level, application of 100 μM NMDA in the presence of low glycine (0.3 μM) and absence of external Mg<sup>2+</sup> induced an inward current which reversed near 0 mV. Co-application of spermine (100-300 μM) increased the NMDA-induced current by about two-fold. Single NMDA-activated channels in outside-out patches were characterized by unitary conductance of 50 pS and an open time distribution that could be fit by a single exponential with a mean open time of 4.5 ms. Perfusion of the patches with 1 μM NMDA (plus 0.3 μM glycine) in the presence of spermine (100-300 μM) increased the open probability of the channels by about 50%, primarily due to an increase in the duration of the openings of the channel. These studies should clarify the mode of action of polyamines on NMDA-activated channels.

464.10

GLUTAMATE RECEPTOR CHANNELS IN RAT DRG NEURONS: SELECTIVE INHIBITION BY LANTHANUM AND GADOLINIUM. J. L. Huettner, Department of Pharmacology, Harvard Medical School, 220 Longwood Ave., Boston, MA 02115

Rat sensory neurons express a novel form of non-NMDA receptor that exhibits strong desensitization to the agonist kainate (NEUROGN 8: 255, 1990). In a search for selective antagonists able to distinguish the receptor in DRG cells from conventional non-NMDA receptors in CNS neurons, we found that lanthanum (La) and gadolinium (Gd) were able to inhibit kainate currents in DRG cells at 100 μM, a concentration that had little effect on kainate current in neurons from cerebral cortex. Blockade of kainate currents in DRG cells by 100 μM La was rapid and complete at holding potentials up to +60 mV. Lower concentrations produced partial block. Full recovery took 60 to 90 sec.

The divalent cations Cu, Mn, Zn, Cd, and Sn were ineffective. Gd was able to produce slight inhibition (5-20% at 100 μM) of kainate currents in DRG neurons but did not reduce the current gated by kainate in cortical neurons.

464.11

NMDA RECEPTOR BLOCK BY H<sup>+</sup>, Mg<sup>2+</sup>, AND Zn<sup>2+</sup> FOLLOWING ALKYLATION OF THE REDOX MODULATORY SITE. Lian-Hong Tong and Elias Aizenman, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have used the alkylating agent N-ethylmaleimide (NEM) to permanently modify the NMDA receptor via its redox modulatory site (Aizenman et al., Neuron, 2:1257, 1990). In addition, we have examined the actions of known endogenous blockers of the NMDA receptor, H<sup>+</sup>, Mg<sup>2+</sup>, and Zn<sup>2+</sup>, following alklylation. The purpose of these experiments is to study any possible relation between the redox site and the sites responsible for block by these various agents.

In cultured rat cortical neurons, NEM (300 μM, 2 min) can effectively "lock" the enhanced-charged NMDA-induced responses produced by the reducing agent dithiothreitol (DTT, 4 mM; 24 min). Under these conditions, the oxidizing agent 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB, 500 μM; 1-2 min) can no longer reverse the potentiating effects of DTT. When we measured the block produced by H<sup>+</sup> (pH 6.8), Mg<sup>2+</sup> (1 mM), and Zn<sup>2+</sup> (20 μM) on NMDA responses before and following alklylation of the redox site (DTT treatment always preceded NEM application), we observed that all three agents could effectively block NMDA responses under both conditions, except that the inhibition produced by Mg<sup>2+</sup> and Zn<sup>2+</sup> decreased slightly after alklylation, while the proton block increased (Δblock alkylated/Δblock control): 1.3, H<sup>+</sup>; 0.8, Mg<sup>2+</sup>; 0.7, Zn<sup>2+</sup>.

Supported by NIH grant NS29365.

464.12


Ethanol's (EtOH) effect on kainate (KA) receptor-operated channels was initially examined in Xenopus laevis oocytes expressing mRNA from rat hippocampal (Em clamped at -70 mV). EtOH (50, 100 mM) significantly inhibited KA-induced currents in the presence of 400, 200, 100, 50, 25, or 12.5 μM KA but did not significantly alter the EC50 for KA (~78 μM). DNXQ inhibited maximum KA responses (400 μM) with an IC50 of ~1 μM; EtOH (50, 100 mM) did not alter the IC50 for DNXQ but did produce further inhibition of KA-induced currents. Despite the apparent noncompetitive inhibition produced by EtOH on KA receptor-mediated responses, the EtOH inhibition of KA-stimulated current increased as the KA concentration decreased. For example, 50 and 100 mM EtOH inhibited 400 μM KA responses by 15 & 25%, respectively, compared to 45 & 51% inhibition of 12.5 μM KA responses. This differential inhibition was not due to different current amplitude stimulated by low vs. high KA concentrations. Oocytes expressing rat cerebellar mRNA also demonstrated greater ethanol inhibition of low vs. high KA responses (e.g. 100 mM EtOH inhibited 12.5 μM KA-stimulated current by 60% compared with 31% inhibition of 400 μM KA responses). However, in oocytes injected with the rat glutamate receptor subunit clone, Glur3, 100 mM EtOH produced similar inhibition of 400 and 12.5 μM KA-induced currents (46 & 52%, respectively). Thus, EtOH's differential inhibition of KA responses in oocytes may reflect selective actions of EtOH on individual KA receptor subtypes.

464.13


The effect of cyanide on NMDA-induced Ca<sup>2+</sup> influx and inward current was studied. [Ca<sup>2+</sup>]<sup>i</sup> was measured in cultured rat hippocampal neurons using fura-2 microfluorimetry. Removal of extracellular Mg<sup>2+</sup> resulted in a five-fold increase in NMDA-induced peak [Ca<sup>2+</sup>]<sup>i</sup>. NACN (1-10 mM) enhanced the peak responses obtained with NMDA in the presence but not in the absence of extracellular Mg<sup>2+</sup>. Similar results were obtained in whole-cell patch clamp recordings from cultured rat hippocampal neurons. Cyanide enhanced NMDA-induced current in the presence, but not the absence of extracellular Mg<sup>2+</sup>. Enhancement of NMDA-activated inward current was observed with intracellular cyanide application. From these results it appears that cyanide alters the Mg<sup>2+</sup> block of the NMDA receptor.

Supported by PHS Grant ES04140.

464.14

Ca IONS CHANGE THE AFFINITY OF GLYCINE TO NMDA RECEPTOR COMPLEX. Yangping Gu* and Li-yen Mae Huang*, Marine Biomedical Institute and Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Ca ion play an important role in regulating the function of NMDA receptor channels. To better understand the interaction between Ca and NMDA receptor channels, we examined the properties of NMDA-activated currents in various external Ca solutions. The whole cell currents were recorded from isolated trigeminal neurons using the patch clamp technique. We found the effects of Ca ions on NMDA-activated currents depend on the concentration of glycine. In the low external glycine, the NMDA-activated currents were increased as we raised the Ca concentration from 2 mM to 20 mM. But in the high external glycine, the NMDA-activated currents were decreased as we increased the external Ca. To study the mechanism of the actions of Ca, we determined the dose response for the effect of glycine on NMDA-activated currents in different Ca solutions. Ca was found to have dual effects on NMDA-activated responses: It increased the binding of glycine to the NMDA receptor complex, and it blocked monovalent cation permeation through NMDA receptor channels.

Supported by grants: John Sealy Memorial Endowment Foundation, RCDA NS01050 and NS23061.
Spermine modulates the activity of NMDA in a variety of receptor binding and electrophysiological studies. At μM concentrations, spermine enhances the action of NMDA but at higher concentrations spermine is less effective. We studied the effect of spermine on NMDA receptor currents using whole cell and single channel recording techniques. Whole cell NMDA currents in cultured rat cortical neurons were enhanced by spermine (0.1 μM - 1 mM) in a concentration-dependent manner. At concentrations above 10 μM, this enhancement was limited to ≈75 nA but not at 75 μM. The percent enhancement of NMDA current by spermine varied among preparations and in some cases no enhancement was observed. In excised outside-out patches, spermine (1 μM - 1 mM) produced a concentration-dependent increase in channel opening frequency with a 40% increase over control at 1 μM. The effect of spermine on opening frequency was independent of external glycine concentration.

These results demonstrate that spermine enhances NMDA current by increasing channel opening frequency. The limitation of enhancement may be due to the other actions of spermine. Spermine is found in high concentrations in the nervous system and appears to act at an important regulatory site on the NMDA receptor-ion channel complex.

This research was supported by the USPHS grant NS19613 (RLM).

L-TRANS L-AMINO-1,3-CYCLOPENTANONE DICARBOXYLIC ACID (ACPD) STIMULATES INTRACELLULAR CA** MOBILIZATION IN A MG** AND DAPV SENSITIVE MANOR IN CULTURED CA1 PYRAMIDAL NEURONS. J. Curry and K.G. Bainbridge. Department of Physiology, University of British Columbia, Vancouver, B.C. V6T 2Z3 Canada.

Trans ACPD has been used to activate a glutamate-g protein linked (GluR2) receptor which is metabotropic rather than ionotropic in nature. This action mobilizes intracellular Ca** and we have studied this effect using the D and L isomers of trans ACPD and monitoring the Ca** levels intracellularly with the dye Fura 2.

Application of the D-isomer produced no effect on neurons at concentrations of ≥1 μM when applied to the bathing medium while the L-isomer produced elevated intracellular Ca** levels at 1 μM. This action of the L-isomer was found to be sensitive to Mg** and the D- and L-amino-5-phosphonoveric acid (DAPV). The results indicate that the L-trans ACPD operates through the N-methyl-D-aspartate rather than the GluR2 receptor and we conclude that the action of the compound may be sensitive to different areas of the CNS and to the age of the neurons being studied.

L-ASPARATE RECEPTORS IN MULLER CELLS: TROPHIC INTERACTIONS. N. Romo-de-Vivar* and A.M. Lopez-Colon. Instituto de Fisiologia Celular, UNAM. Apartado Postal 70-600, 04510 México, D.F., México.

Receptors for L-N-aspartate have been biochemically characterized in Muller cells from the chick retina in primary culture. Two binding sites with affinity in the μM and μM range were detected, showing pharmacological profiles similar to those for neurotransmitter (QA) and high-affinity uptake systems, respectively. Since these receptors became apparent at different stages of development in vitro, we tried to determine if culture conditions could influence their expression or promote new properties. Glial cells were maintained in medium containing 10% FCS. The effect of low sérum (1%), ddOA, L-glutamate, quisqualate and DAPV was studied on the aspartate binding. At days 1,5,8 and 12 in vitro. At concentrations 50-200 μM, neither compound showed effect; ddOA was tried up to 1 μM without effect in the cultured in low sérum. L-N-lasp binding was increased at all stages: 0.497 to 0.762 pmole/mg protein at 1 DIV, 0.123 to 1.05 at 5 DIV, 0.190 to 1.20 at 8 DIV and 0.24 to 0.82 at 12 DIV. Pharmacologically these sites show the properties of high affinity transport sites. These results suggest that in contrast with cells in culture, Muller cells in low sérum do not express high affinity binding sites with properties similar to QA transmitter receptors.
GYR IPSP (84%; n=5). The glutamate evoked IPSP from PYR showed little sensitivity (6%, n=2) to the GABAB antagonist 2-OH-saclofen (1 mM). In one cell the glut-IPSP (116% of control). Repetitive electrical stimulation (3 Hz for 10 sec; the mean amplitude of the glut-IPSP was -2.0 mV and the mean latency was increased by only 26.3 ± 12.9% (n=7) by the 103rd test pulse. In the presence of baclofen (100 μM), the amplitude of the 103rd response was decreased by only 23.1 ± 13.0% and the latency was increased by only 20.1 ± 4.1% (n=6).

Our results demonstrate that: (1) functional GABA-A and -B receptors coexist in the neonatal rat optic nerve; (2) baclofen improves long-frequency impulse conduction in the myelinated optic nerve. For example, with the presence of 100 μM Ca2+ and 2 mM Mg2+ in the perfusate and in 20 cells, the mean amplitude of the glut-IPSP was -2.0 mV and the mean latency was increased by only 26.3 ± 12.9% (n=7) by the 103rd test pulse. In the presence of baclofen (100 μM), the amplitude of the 103rd response was decreased by only 23.1 ± 13.0% and the latency was increased by only 20.1 ± 4.1% (n=6).

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465.7


D-baclofen blocks the action of L-baclofen in the trigeminal nucleus and spinal cord but not in neocortical or hippocampal slices. This discrepancy could be due to a difference in baclofen concentration used in the two assays.

The iontophoretic administration of the GABA antagonist 2-hydroxy-saclofen and CGP 35348 blocked the action of iontophoretically applied L-baclofen on neurons in the trigeminal nucleus of rats anesthetized with halothane, with CGP 35348 being 3-4 times as potent as saclofen.

The iontophoretic administration of GABA resembled L-baclofen in depressing excitatory transmission and facilitating segmental inhibition in the trigeminal nucleus. CGP 35348 also blocked the depression of excitatory transmission and partially blocked the facilitation of segmental inhibition produced by GABA.

Our observations indicate that CGP 35348 is not only a baclofen antagonist but actually a GABA receptor antagonist, and that baclofen is acting at GABA receptors in the trigeminal nucleus. The portion of the GABA effect not blocked by CGP 35348 is probably mediated by GABA receptors, since we have previously found that segmental inhibition in the trigeminal nucleus can be modulated by GABA agonists and antagonists. (Supported by NS-19889)

465.9


The development of GABA activity in the dentate gyrus was studied by examining GABA-evoked, antidromically-evoked field potentials. The effects of GABA receptors on peptide release from primary neurons, the granule cells, occurs.

Without being present before the peak generation of GABA's primary neurons, the granule cells, occurs.

465.11


Recently developed partial agonists at the benzodiazepine receptor may be less sedating than the classical full benzodiazepine agonists. This study was therefore designed to evaluate the changes produced in the frequency spectrum of the cortical electroencephalogram (EEG) in the rat as a way of quantifying the sedative properties of these compounds. RO16-6028 (1.0, 0.25, 0.03 mg/kg) and CGS 9896 (1.0, 0.2 mg/kg) were thus compared with Diazepam (0.5, 0.04 mg/kg) and Alprazolam (0.125, 0.02 mg/kg/kg). Rats were chronically implanted with cortical electrodes, and ipsilateral fronto-occipital EEG was recorded in all cases. Drugs were administered via a carotid catheter. The EEG was visually scored according to the等级, and drug effects were evaluated for up to an hour following compound administration. Vigilance was maintained throughout the experiment, and the ability of the EEG to reflect any changes in the EEG was calculated. All compounds at doses increased power in the 8 to 30 Hz frequency band, which has been suggested to represent idling in thalamo-cortical circuits. The EEG change that is consistent with the anxiolytic action of the benzodiazepine receptor antagonist for the two major GABA receptors or agonists: the benzodiazepine receptor agonist (Flumazenil) and the GABAB receptor antagonist (CGS9896). All new compounds in the benzodiazepine receptor antagonist group were above the level of the benzodiazepine receptor antagonist, and the benzodiazepine receptor agonist (Flumazenil) was above the level of the benzodiazepine receptor antagonist. This supports the idea that the benzodiazepine receptor antagonist (Flumazenil) is more specific for the GABAB agonist than for the benzodiazepine receptor antagonist.

The discriminative-stimulus effects of specific agonists for the two major GABA receptors studied in rats trained to discriminate either the GABA agonist THIP (gabab-covered) or the centrally occurring GABA agonist muscimol or the GABA agonist baclofen (a central muscle relaxant) from the no-drug condition. Discrimination performance was achieved more rapidly under the THIP (5.6 mg/kg, i.p.) than under the baclofen (3.2 mg/kg, i.p.) training condition. In tests, there was dose-dependent generalization to agonists of the training drugs, with each drug being specific in that baclofen did not occasion drug-lever responding reliably in rats trained to discriminate THIP and vice versa. Administration of the specific benzodiazepine-receptor antagonist flumazenil dose-dependently decreased drug lever responding in combination with THIP but not in combination with baclofen. The baclofen training condition per se was less specific than the THIP training condition, because rats trained to discriminate baclofen were more likely to generalize to midazolam (MDZ) and to pentobarbital (PB) than were rats trained to discriminate THIP. The effects of MDZ were determined by the GABA receptor. Thus, a major part of the baclofen/GABA discriminative-stimulus complex may be a generalized muscle-relaxant effect. (Supported by NIDA Grant DA4133)

465.10


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645.13

466.3 NEONATAL OPIATE ABSTINENCE IN THE ABSENCE OF TOLERANCE. B. T. Windh, P.J. Little and C. M. Kuhn. Dept. of Pharmacol., Duke Medical Center, Durham, NC 27710.

We have demonstrated that neonatal rats are more refractory to developing opiate tolerance to morphine analgesia than are weanling rats. Therefore, neonatal rats provide a useful model for addressing the question of the obligate association of opiate tolerance and abstinence. The following work was performed to determine whether the same chronic morphine regimen which did not cause neonatal tolerance to analgesia can result in abstinence. Rats were treated with 5-25 mg/kg morphine (X/day, s.c.) from 4-8 days of age. Animals were killed 12 h after the last injection. There were found no differences in the withdrawal score of opiate withdrawal (e.g., diarrhea, salivation, tremor) between controls and morphine treated rats. These results suggest that additional mechanisms and sites of action (e.g. in the kidney cortex) are involved.

466.5 SPINAL CORD OPIOID PEPTIDE LEVELS ARE ELEVATED DURING GESTATION. V.M. Medina* and A.R. Gintzler. Dept. of Biochem., SUNY Health Science Ctr., Brooklyn, NY. 11203.

In laboratory animals and humans, pregnancy is associated with an opioid receptor-mediated increase in the threshold for maternal responsiveness to aversive stimuli. Pharmacological and behavioral experiments indicate that, at least in part, this analgesia results from the activation of a spinal cord dynorphin/kappa opioid receptor system. We now demonstrate that during late pregnancy there is a significant elevation in the content of opioid peptides in specific regions of the rat spinal cord. Opioid content was assessed by RIA using antibodies selective for either dynorphin (1-17 or 1-8) or met-enkephalin. On day 22 of gestation, in 5 out of 5 rats, the content of dynorphin (1-17) and dynorphin (1-8) increased by 35 and 60%, respectively in the lumbar spinal cord (p < .01). In contrast, levels of both these opioid peptides did not show any consistent change in the cervical or thoracic spinal cord at this stage of pregnancy. Levels of met-enkephalin increased in the lumbar and thoracic cord (65% and 32%, respectively; p < .05) in 4 out of 5 pregnant (day 20) rats. In addition, there was a numeric, but not statistical, increase in met-enkephalin content in the cervical region. These results provide the first biochemical evidence that during gestation, there is a positive modulation of spinal cord opioid systems.

466.6 ENDGENOUS OPIOID PEPTIDES (EOP) ARE INVOLVED IN THE SUCKLING INDUCED PROLACTIN INCREASE IN FEMALE RATS. P. Callahan, M. Zanimal and J. Jank. Miami University, Oxford, OH 45056.

The purpose of these experiments was to identify the specific EOP involved in the suckling induced prolactin secretory response. Post-partum, lactating female Sprague-Dawley rats were used for all experiments. Animals were implanted with chronic intraventricular (ivt) cannulae into the lateral ventricle of the brain. Following a 5-7 day recovery period and one day prior to an experiment, each animal was implanted with a chronic jugular cannula. On the day of the experiment, pups were separated from their dams and each animal was given either saline or antiserum to beta-endorphin, met-enkephalin or leu-enkephalin (0.75 or 1.5 ug, ivt in Saline) 30 minutes prior to sacrificing. Thus, the suckling induced prolactin increase was markedly attenuated in each treatment group compared to control animals. These results clearly demonstrate that beta-endorphin, met-enkephalin and leu-enkephalin are involved in the mediation of prolactin release in female rats.

466.7 EFFECTS ON NEUROHYPOPHYSIAL HORMONE RELEASE, DIURESIS AND ELECTROLYTE OUTPUT OF \(\mu\) AND \(\kappa\)-OPIOIDS. B. van de Hauing & T.F. van Wimersma Greidanus*. Rudolf Magnus Dep't of Pharmacology, Duke Medical Center, Durham, NC 27710.

Opioids alter fluid metabolism and electrolyte balance. The mechanism of action is assumed to involve opioid modulation of neurohypophysial hormone release. The effects of the \(\kappa\)-agonist U69,593 and the \(\mu\)-agonist DADLE on urinary output and renal electrolyte handling were investigated in normally-hydrated, non-fluid-loaded, male rats. Using a protocol of hourly urine sampling up to 6 h, it was found that the s.c. applied \(\kappa\)-agonist (dose range 0.01-5 mg/kg) induced a prominent diuresis up to 3 h post injection. The diuretic effects of the \(\mu\)-agonist did not affect urination but inhibited sodium output. Therefore, these results provide the first biochemical evidence that during gestation, there is a positive modulation of spinal cord opioid systems.
467.1 EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION OF A CANINE 5-HT1D RECEPTOR SUBTYPE. J.M. Zombick, R.L. Weinshank, M. Machacek, T.A. Behan, S. Dabbaghian, and P.R. Harrel. Neurogenetic Program, Paramus, N.J. 07652

RDC4, a guanine nucleotide-binding protein (G protein) coupled receptor originally defined in a neuroblastoma cell line by Luber and colleagues, was identified as the canine homolog of the human 5-HT1D receptor. Clone RDC4 is an intronless gene encoding a protein of 377 amino acids containing three consensus sequences for N-linked glycosylation in its amino terminus. The deduced amino acid sequence of clone RDC4 exhibits greatest sequence identity (43%) with the 5-HT1D receptor and lower overall homology for the amino and carboxyteric receptors. This sequence information suggested that clone RDC4 encoded a novel serotonin receptor. In order to determine the pharmacological identity of clone RDC4, murine LM (tk) fibroblasts were stably transfected with the DNA encoding this gene and radioligand binding studies were conducted using [3H]-HT binding sites. Membranes prepared from stable transfectants displayed an apparently homogeneous population of high affinity (Kd = 3.6 nM). Stable (Bmax = 27 fmol) protein ([3H]-HT binding sites. High affinity ([3H]-HT binding was unchanged using assay conditions (1 μM pindolol and 1 μM SCH 23390) for pharmacologically mask 5-HT1, 5-HT2, and 5-HT3 receptors. Serotonin ligands displaced specific ([3H]-HT binding with a rank order of potency expected of a 5-HT1 receptor subtype: SHT > yohimbine > DPAT > Ketanserin > Spiperone > Zocapride. These binding properties are supportive of the designation of clone RDC4 as a 5-HT1 receptor subtype.

467.2 IDENTIFICATION OF A NOVEL G PROTEIN-COUPLED RECEPTOR WHICH EXHIBITS HIGH HOMOLOGY TO CLONED SEROTONIN RECEPTORS. Y. Shen, F.J. Moreira, Jr., C.B. Gerfen, L.C. Mahan*, P.A. Jose, M.M. Mouradian & D.R. Sibay. Experimental Therapeutics Branch, NINDS and Laboratory of Cell Biology, NIH, NIH, Bethesda, MD 20892

We have utilized the pig δ-chain receptor (PCR) technique to selectively amplify G protein-coupled receptor cDNA sequences from rat kidney proximal convoluted tubule (PCT) mRNA. Poly (A) mRNA was synthesized and reverse transcribed followed by PCR amplification using sets of highly degenerate primers derived from the transmembrane sequences of previously cloned dopaminergic, adrenergic, and serotonergic receptors. This process resulted in the amplification of a number of discrete cDNA fragments. Sequence analysis of some of these fragments revealed several previously unidentified receptors as well as some putatively novel receptor cDNA sequences. One cDNA was identified which, when compared to all G protein linked receptors cloned thus far, appears to be most closely related to the serotonin receptor family. Within the transmembrane domains, the rank order of homology is 5HT4 > 5HT2 > 5HT3. Northern blot analysis reveals a 3.6 kb transcript with the following rank order of abundance in CNS tissues: hypothalamic > hippocampus > mesencephalon > olfactory bulb > cerebral cortex > olfactory tubercle > striatum. In peripheral tissues, this mRNA is most abundant in the spleen with lesser amounts seen in the kidney, ovary, lung and uterus. In contrast, this transcript is not detectable in the cerebellum, pituitary, retina, testes, stomach, prostate, skeletal muscle, liver, gut or heart. In situ hybridization analysis confirms the Northern blot data and also reveals a high level of transcript in the thalamic reticular nucleus. Full length cDNA clones have been isolated and are being expressed to establish the specific pharmacology and function of this novel receptor subtype.

467.3 5-HYDROXYTRYPTAMINE BLOCKS GABA, BUT NOT GABA, POST-SYNAPTIC POTENTIALS IN Dopamine NEURONS. S.M. Johnson, R.B. McCoury and R.A. North. Vollum Institute, 3181 SW Sam Jackson Park Rd., Portland, OR 97201.

Intracellular recordings were made from presumed dopamine neurons in slices cut from the midbrain of the rat. Focal electrical stimulation produced a lasting long-lasting hyperpolarization (1-2 mV), which was blocked by the GABA receptor antagonist 2-hydroxy-saclofen (100 μM). Serotonin (5-HT; 3-100 μM) reduced the amplitude of this synaptic potential by 20-84% with an EC50 of 10 μM, but did not reduce the amplitude of synaptic potentials mediated by GABA receptors. The effect of 5-HT was not affected by the 5-HT1 agonist 1-(3-(trifluoromethyl)phenyl)piperazine (300 μM), but not by the 5-HT1 agonist N-dipropyl-5-carboxamidotryptamine (1 μM) or the 5-HT2 agonist (g)-2-(5-dimethoxy-1-isodopamine)-2-amino-propane (10 μM). Cyano-pindolol (30 μM), a 5-HT2 antagonist, blocked the effect of 5-HT. Hyperpolarizations produced by superfused GABA (1 μM) were not affected by 5-HT (30 μM), suggesting that 5-HT does not act post-synaptically. We conclude that 5-HT activates presynaptic 5-HT1 receptors which inhibit the release of GABA onto GABA, but not GABA, receptors.

467.4 FUNCTIONAL SUBSENSITIVITY OF SEROTONIN RECEPTORS MEDIATING CLONIDINE-INDUCED INCREASES IN GROWTH HORMONE IN FAWN-HOODED RAT STRAIN RELATIVE TO WISTAR RAT STRAIN. C.S. Auda and L. H. Weisshank* and C. L. Murphy* Lab of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Brain serotonin changes have been implicated in the etiology of affective illness and the mode of action of antidepressant and anxiolytic drugs (Mezey et al., 1995; Halpin et al., 1997). In the present study, we investigated the effects of various serotonin receptor subtype selective antagonists (mesulergine, metergoline and others) on clonidine-induced hypactivity and increases in growth hormone levels in male Wistar rats. In addition, we compared the effects of various doses of clonidine on growth hormone levels and locomotor activity in male and female Wistar rats. Pretreatment with mesulergine and metergoline, did not affect clonidine-induced hypactivity but completely blocked clonidine-induced increases in growth hormone levels. Furthermore, clonidine-induced increases in growth hormone levels were significantly less in the FH rats relative to Wistar rats. On the other hand, clonidine-induced hypactivity was not significantly different between these two rat strains. These findings support other evidence indicating that FH rat strain may prove to be a useful genetic model for some brain disorders with possible abnormalities in serotonergic function such as depression, obsessive-compulsive disorder, eating disorders and other neuropsychiatric disorders.


Rats of the Fawn-Hooded (FH) strain exhibit a hemispheric disorder known as platelet storage pool deficiency. Behavioral studies indicated that serotonin function in the CNS is altered in this rat strain relative to Sprague-Dawley (SD) animals. In addition, FH rats drink excessive ethanol and display symptoms related to an animal model of depression compared to control animals. In the present study, specific brain regions from FH, SD and Wistar rats were examined for differences in 5-HT brain receptors. [3H]-8-OH-DPAT was used to label 5-HT1A receptor sites and the KD values for frontal cortex, hippocampus and striatum were similar in all three rat strains. However, the Bmax values for [3H]-8-OH-DPAT binding in the striatum of FH rats were significantly lower than the SD and Wistar rats. [3H]-8-OH-DPAT may be labelling presynaptic autoreceptors in the striatum. Preliminary results from 5-HT1D receptor studies using rat antisera to label the binding sites indicate that there are no significant differences in KD values in these brain regions comparing the three strains, but the Bmax values for the cortex and striatum were significantly higher in FH compared to SD rats. Ongoing autoradiographic studies and comparison of mRNA levels will provide further information on these 5-HT receptor differences.
CALMODULIN ANTAGONISTS INHIBIT TRITIATED IMIPRamine BINDING TO THE SEROTONIN UPTAKE APPARATUS. D.M. Helmote and S.W. Goldberg. Dept. of Pharmacology, Long Beach, CA 90822. Dept. Psychiatry, University of California, Irvine CA 92715, USA.

In an effort to evaluate potential ligands for the tritiated imipramine (IMI) binding site, we examined the effects of several calmodulin antagonists. The IMI binding to the 5HT uptake complex. It has previously been found that calmodulin antagonists reduce the in vitro uptake of and recovery of precursors to neurotransmitter systems. Specifically we found that calmodulin antagonists with high affinity for calmodulin (e.g., W-7, calmidazolium, and calmidazolium type structures inhibited the 3H-IMI binding to either 5HT1A, 5HT1B, and 5HT1C receptors. Competition studies were performed in rat cerebral cortex and calf cortex using 3H-IMI and 5HT1A, 5HT1B, and 5HT1C receptors. The binding inhibition was not affected by the presence or absence of EDTA or calcium. This is inconsistent with an action on calmodulin since these antagonists require calcium for their interaction with this protein. Preparations of all three receptors bound to calcium. This is inconsistent with an action on calmodulin since these antagonists require calcium for their interaction with this protein.

EVIDENCE THAT THE SEROTONIN RELEASER P-CHLOROAMPHETAMINE ELEVATES BLOOD PRESSURE, PLASMA RENIN AND PLASMA CORTICOSTEROIDE THROUGH CENTRAL MECHANISMS. Ritterhouse PA, Lexy AD, Li Q, Yracheta JM, Kunimoto K, Van de Kar LD. Loyola University Chicago, Maywood, IL 60153.

PCA is a serotonin (5-HT) releaser that reliably increases both plasma corticosterone (COR) and plasma renin concentration (PRC) when injected peripherally. The aim of these studies was to determine the role of central 5-HT receptors in mediating this hormonal response. PCA was injected intraventricularly in male rats at doses (50, 100, 1000 μg/kg) below those peripherally effective. Trunk blood was collected 1 hour later. CORT was elevated in a dose dependent manner, while PRC did not change. Pretreatment with the 5-HT1A antagonist LY53857 (20 μg/kg, ICV) did not affect either the CORT or PRC response. Since an increase in blood pressure (BP) due to PCA could inhibit the PRC response, arterial cannulators were implanted to record BP. We found that 1.0 mg/kg PCA significantly raised BP (p < 0.01) at 2 and 5 minutes after ICV injection, but not after peripheral intra-articular injection. An additional experiment showed that a dose of 5-HT (100 μg/kg, ICV) significantly increased PRC (p < 0.01) if preceded by the 5-HT1A antagonist prazosin (1.0 mg/kg, sc). Thus, by masking the cardiovascular effects with prazosin, PCA’s stimulatory effect on renin was exposed. Finally, ibotenic acid lesions were made in the hypothalamic paraventricular nucleus (PVN). Ibotenic acid destroys cell bodies, but leaves fibers of passage intact. Two weeks after surgery rats were injected with 8 μg/kg PCA (p). Rats with histologically verified PVN ibotenic acid lesions had significantly attenuated CORT (p < 0.01) and PRC (p < 0.05) responses compared to vehicle treated controls. Thus, cell bodies in the PVN mediate the CORT and renin response to PCA. Supported by AHA, Chicago.

NOVEL HIGH AFFINITY 3H-SEROTONIN BINDING SITES IN BRAIN TISSUE:EVIDENCE FOR FURTHER SHT, RECEPTOR SUB-TYPES. E. Weisberg and M. Teitler. Dept. Pharmacology and Toxicology, Albany Medical College, 47 New Scotland Avenue, Albany, New York 12208.

We report herein that high affinity 3H-SHT binding homogenates of bovine and rat caudate and cortical tissues cannot be interpreted without including additional 'SHT-like' receptors other than the characteristic SHT1A, SHT1B, SHT2A, and SHT2B receptors. Competition studies were performed in rat and bovine frontal cortex and caudate tissues, using pharmacological characterization by SHT1A, SHT1B, and SHT2A receptors. The results suggested that SHT receptors could be further characterized by cholinergic, dopaminergic, and GABAergic mechanisms.


We have demonstrated that SHT inhibits, via SHT1-like receptors, the spontaneous rhythmic constriction (SRC) and KCL-or U46619-induced sustained active muscle tone (SAT) of porcine pial veins. In this study, we further characterized the SHT receptors mediating inhibition of SRC and KCL-or U46619-induced SAT of porcine pial veins, using an in vitro tissue bath technique. Changes in isometric vessel wall tension were measured. The results indicated that e-NOS, SHT1A, and SHT1B agonists, induced a small enhancement of SRC and SAT. 2-NE-SHT, a SHT1A agonist, did not affect SRC and SAT. 8-OD-PAT (a SHT1A agonist), TPMP (a SHT1A agonist), and 5-methoxytryptamine (5-HT1A, 5-HT1A agonist) inhibited SRC and SAT in a dose dependent manner. The Emax values, SHT1 is the most potent preferential 5-HT1A agonist inhibiting SRC and SAT. On the other hand, GR317158 (a SHT1B agonist) did not affect SRC or KCL. Ketanserin, a 5-HT1 antagonists, did not significantly affect the SHT-mediated inhibition of SRC and SAT. Results of this study indicate that multiple SHT receptors are involved in 5-HT inhibition of SRC and SAT in porcine pial veins, although 5-HT1 antagonists appears to be most potent in inhibiting these contractions.
ORAL TRACT: THE LOCALIZATION OF 5-HT3 RECEPTORS IN THE TONGUE AND Oesophagus

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5-HT3 receptors are localized on the epithelial cells of the oral cavity, including the tongue and oesophagus. These receptors are involved in various physiological processes such as salivation, taste, and the perception of pain. The localization of 5-HT3 receptors in the tongue and oesophagus is important for understanding the role of these receptors in the processing of sensory information from the oral cavity.

The localization of 5-HT3 receptors in the oral cavity was conducted using immunohistochemical methods. The localization of 5-HT3 receptors was detected using antibodies specific for 5-HT3 receptors. The results showed that 5-HT3 receptors are localized on the epithelial cells of the tongue and oesophagus. The localization of 5-HT3 receptors was observed in the oral cavity, including the tongue and oesophagus. The results showed that 5-HT3 receptors are localized on the epithelial cells of the tongue and oesophagus. The localization of 5-HT3 receptors was observed in the oral cavity, including the tongue and oesophagus. The results showed that 5-HT3 receptors are localized on the epithelial cells of the tongue and oesophagus.
468.1 ACTIONS OF BUTYRFENINE ON THE STORAGE AND RELEASE OF SEROTONIN (5-HT) FROM RAT SPINAL CORD SYMPATOHES. P.J. Monos, D.L. Smith, G.M. Williams, and D.L. Auerbach. Dept. of Neurosurgery, West Virginia University Health Science Center, Morgantown, WV 26505.

The 5-HT3 receptor agonist butyrfenine was found to evoke a dose-dependent (100 nM - 10 μM) increase in uptake of [3H]-5-HT, with no effect on the 5-HT1b receptor system. These data suggest that butyrfenine causes an increase in the storage of 5-HT.

468.2 BIOCHEMICAL EFFECTS OF EXPOSURE TO NITROUS OXIDE (N2O) ON RAT BRAIN NORMONAMINES. D. Oromanouili, Z. Papadopololou, Daffoul, L. Koral**, and Univ. Illinois Col. Med. at Rockford, IL 61017, U.S.A.

N2O is reported to influence brain monoaminergic transmission (Passino et al., Soc. Neurosci. Abst. 13:913, 1987). To further explore possible effects of N2O upon brain monoamines, male Sprague Dawley rats, 220 ± 20 g, were individually exposed to either 70% N2O or room air for 15 min then sacrificed. The frontal cortex, striatum, hippocampus and hypothalamus were dissected for measurement of the monoamines dopamine and 5-hydroxytryptamine (5-HT) and metabolites DOPEG, 5-HIAA and 5-HIAA using ICP/ICP with a radiochemical detection. Our results show that N2O exposure significantly raised levels of 5-HIAA and increased the 5-HIAA/5-HT ratio in the hypothalamus but lowered the 5-HIAA/5-HT ratio in the frontal cortex due to elevated levels of 5-HT. Recent work implicating an action of N2O on benzodiazepine and perhaps GABA mechanisms (Izamal and Quock, 1988) may be relevant to the changes of 5HT systems after N2O exposure. (Supported by N.I.H. Grants DE-06894 and DE-09378.)


PCPA is a potent and selective inhibitor of TPH, the first and rate-limiting enzyme in the biosynthesis of serotonin. Thus, it might be a useful tool for studying the regulation of TPH gene expression. In the present study we assessed the time course and magnitude of the changes in TPH activity and mRNA as well as that of its product, serotonin (5-HT) in three brain regions, the dorsolateral striatum, hippocampus (H) and cerebral cortex (CB). PCPA (300 mg/kg) was injected subcutaneously and rats were killed at 12 hrs, 1, 2, 4, and 7 days post-treatment. TPH mRNA in the DRN, demonstrated by in situ hybridization, increased almost 2-fold by 1 day post-PCPA and returned to control levels by 4 days. In contrast, TPH activity in the DRN, H and CB was reduced to about 10% of control levels 1-2 days post-treatment, returning to control levels by 2 weeks. The rise and return of 5HT immunostaining in fibers followed the same time course as TPH activity. No effect was observed on aromatic-L-amino acid decarboxylase activity, immunoreactivity or mRNA levels. These data suggest that inhibition of TPH by PCPA-treatment results in the specific up-regulation of TPH mRNA expression and new enzyme synthesis.


The activity of tryptophan hydroxylase (TPH), the initial and rate-limiting enzyme in the biosynthesis of serotonin, is increased by Ca2+-calmodulin-dependent protein kinase, presumably as a result of phosphorylation of TPH. Several studies suggest that cAMP also activates TPH, but a role for protein kinase A (PKA) in this process has not been established. Concentrations of cAMP, dibutyryl-cAMP and 8-bromo-cAMP which activate endogenous PKA did not alter TPH activity in the striatum, hippocampus or pinal gland, even when purified activator protein (protein 14-3-3) was added to the assay. Similarly, the catalytic subunit of PKA failed to activate TPH. To determine whether TPH is phosphorylated by PKA, crude extract from rat striatum, tegumentum, and pinal were incubated with 32P-ATP (2 μClube). Proteins were subjected to SDS-PAGE and Western blotting, and autoradiograms were produced from the blots. TPH, identified on blot with a monoclonal antibody (PHB), was excised and its 32P content was determined by scintillation counting. The results clearly show that 20 μM cAMP or 1 μg purified PKA catalytic subunit increase the phosphorylation of TPH in each brain area. The cAMP-mediated phosphorylation of TPH was inhibited by 50 μM of the PKA inhibitor H-7. These results indicate that PKA phosphorylates TPH, however this phosphorylation does not appear to produce an increase in catalytic activity.


Fibers immunoreactive for TPH, the synthesizing enzyme for serotonin (5-HT), have been visualized around cerebral blood vessels (Neurosci. Lett., 1990, 116:269-274). In order to determine their origin, rats were subjected to bilateral removal of the superior cervical ganglion though in adjacent sections, cells were immunoreactive for serotonin. Supported by NIH Training Grant 5 T32-GM07039 and WVU Dept. of Anesthesiology.


A role for serotonin (5-HT) in CNS modulation of prolactin (PRL) surges is supported by physiological and pharmacological experiments. We have used microdialysis and peripheral blood sampling concurrently in unrestrained lactating rats to reexamine the possibility that 5-HT exerts a PRL releasing effect. Dialysis probes were implanted in the anterior preoptic area (APoA) of lactating female rats. A 90 minute suckling bout, following 6 hours of pup separation, significantly increased PRL levels at 30 minutes to 215 ± 26 ng/ml (mean ± s.e.m., n=3) from a baseline of 6 ± 1 ng/ml, and levels remained elevated while the pups were present. In contrast, extracellular 5-HT release in the APoA was unchanged during suckling.

The APoA had been incubated as a PRL releasing site in earlier 5-HT turnover studies, so we verified probe placement in the hypothalamus by locally infusing the 5-HT releasing agent, 5-HTP (30 μg). This resulted in a large increase in 5-HT (5337 ± 698 μg/ml) and PRL (118 ± 25 ng/ml from a baseline of 9 ± 1 ng/ml) at 30 min.
648.7 EXCITABILITY IS DECREASED DURING THE SEROTONIN-INDUCED SLOW DEPOLARIZATION OF MUPPUPID CARDIAC NEURONS. R.L. Parsons, I.M. Konopka and J.C. Herdwick. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Many small intrinsic neurons in the muppid cardiac ganglion exhibit 5-HT immunoreactivity. Local application of 5-HT produced a biphasic depolarization in approximately 50% of the muppid parasympathetic postganglionic neurons responding to 5-HT. This biphasic 5-HT-induced depolarization was comprised of a fast, brief component followed by a slower, longer lasting period of depolarization. The fast depolarization was consistently accompanied by a decrease in membrane resistance (R_m). The slow 5-HT-induced depolarization was accompanied by an increase in R_m in 14 cells and a decrease in R_m in 12 cells. During the 5-HT-induced fast depolarization, action potentials occurred often were increased. In contrast, during the slow 5-HT-induced depolarization, no spiking occurred regardless of whether R_m decreased or increased. Application of long duration suprathreshold depolarizing current pulses to the parasympathetic neurons initiates multiple action potential activity. During the 5-HT-induced slow depolarization, fewer spikes were produced by the application of identical depolarizing current pulses. These results show that during the 5-HT-induced slow depolarization membrane excitability was depressed. Supported by NIH Grants NS-29787 and NS-25973.


GFAP mRNA levels were measured by Northern blot analysis in different regions of rat brain to get information on potential neurotoxicity of drugs on central serotonin-5-HT-containing neurons. Intracerebroventricular administration of 5,7-dihydroxytryptamine (150µg/20µl) 30 min after 25mg/kg intraperitoneally of desipramine, to protect noradrenergic nerve terminals, caused a significant increase of GFAP mRNA levels in the hippocampus but not in the striatum, 5 days later. Intraperitoneal administration of d-fenfluramine (10 mg/kg b.i.d. for 4 days), a potential neurotoxic agent for 5-HT neurons, caused similar decreases of 5-HT in hippocampus (about 80%) and striatum (about 60%) but did not change GFAP mRNA levels in either region. Since 5-HT has been found to exert an inhibitory influence on GFAP synthesis in brainstem astrocytes culture (Le Prince et al. Dev. Brain Res. 1990,51,295), it is not clear whether the changes of GFAP mRNA levels were secondary to modifications in the serotonergic control of GFAP synthesis, to astrocytes hyperpolarization or both.

648.9 D-LYSERGIC ACID DIETHYLAMIDE (LSD) POTENTLY ENHANCES A HYPERPOLARIZATION-ACTIVATED CATION CURRENT (I_h) IN FACIAL MOTOR NUCLEUS (FMN) NEURONS. J.C. Gurney and G.K. Achajian. 1,2. Dept. of Pharmacology, 1 and Psychiatry, Yale University. New Haven CT. 06508.

The facial motor nucleus has a density of binding sites and mRNA for 5-hydroxytryptamine (5-HT) receptors. 5-HT has been shown to induce a slow depolarization/inward current in FMNs via 5-HT receptors. In the present study, the effects of LSD on components of the 5-HT response, i.e. depolarization and an increase in a hyperpolarization-activated cation current (I_h) were examined in an in situ slice preparation of rat facial motor nucleus by current clamp and voltage clamp methods. 5-HT increased the electrical excitability of FMNs and induced a slow depolarization. LSD (10 µM) produced a larger increase in the excitability of FMNs but, in contrast, produced little or no inward current and progressively blocked the depolarization produced by 5-HT.

Since LSD produced an increase in excitability which was more current led us to examine the effects of LSD as well as 5-HT on voltage-dependent currents. 5-HT was found to produce a small enhancement of I_h current in FMNs. Interestingly LSD (100µM) was more efficacious than 5-HT at increasing this current. The effects of LSD on I_h persisted over 1hr, with maximal effect seen after 20 mins. The reversal potential for I_h was -30 mV, thus having the characteristics of a mixed Na/K+ channel. The 5-HT antagonists, 1,2-dimethoxy-4-methylphenyl-2-amino-propane (DOM) and (2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI) also enhanced I_h in a degree intermediate between 5-HT and LSD.

Our findings suggest that LSD has dual actions in FMNs: 1) as a full agonist on an I_h current (2) as a low efficacy partial agonist on the 5-HT induced inward current.

Biochemical (HPLC-UV detector) and immunocytochemical (HRP) techniques were used to study the GABAergic and serotoninergic neurotransmitter systems in the superior colliculus (SC) of adult and aging rats. GABAergic and serotoninergic neurotransmitter systems in the superior colliculus (SC) of adult and aging rats were analyzed according to the slightly modified method of Maldonado et al. (1989) using a precolumn derivatization technique with Phenylthiohydantoin as the tagging reagent using Pico-tag and UV detection. To determine the amount of neurotransmitters, 70 μm serial sections of brain tissue fixed with a mixture of aldehydes- were processed for GABA or serotonin immunohistochemistry and were analyzed with light microscopy. GABA concentration did not show statistically significant changes in aging. However, since there was a significant decrease of the weight of the SC in aged rats, there is an age-related loss of GABA in the SC. This finding is consistent with a conspicuous decrease in the number of GABA immunoreactive neurons in aged rats. Likewise, serotonin immunoreactive fibers showed networks of remarkably swollen varicosities in aged rats, suggesting a possible axonal degeneration.

At spinal synapses SHT and TRH act as co-transmitters by blocking Ca2+ currents of rat hippocampal cells. In isolated rat hippocampus slices (30 min superfused with Krebs' solution) SHT elicited an outward current (0.15 ± 0.03 nA) associated, in 50% of neurons, with a 27 ± 3% fall in input resistance. Depolarizing steps activated slow K+ currents which, after peak, decayed slower than in control conditions (P < 0.05). Inhibitory effects of TRH were found to be depressed (35% at -20 mV) by SHT. TRH (10 μM) had little effect on steady current or input resistance, but it reduced by 18% (-20 mV) the slow K+ current. The application of SHT and TRH gave smaller (40%) reductions in this current than those predicted by the sum of the effects of the two drugs. The slow K+ current remaining in SHT -free solution was identified as the delayed rectifier (I_d) which was attenuated by 9% and 26% in the presence of SHT or TRH, respectively. Co-application of these agents did not change I_d.

We suggest that since SHT diminished the total slow K+ current (mainly by blocking its Ca2+-sensitive component) this action would reduce the efficacy of inhibitory K+ conductance systems. TRH appeared to block the effect of SHT on the slow K+ current, suggesting a modulatory role of this peptide in some excitatory actions of SHT present at depolarized membrane potentials.


Ethanol (ET) has previously been shown to increase extracellular levels of serotonin (5-HT) and dopamine (DA) in the nucleus accumbens (NA). To determine if this effect is regionally specific, levels of these neurotransmitters and their metabolites were measured in the lateral hypothalamus (LH) and NA concurrently using in vitro microdialysis followed by HPLC-EC, prior to and after intraperitoneal injection of ET. Male Wistar rats were injected with vehicle or ET (0.5 or 1.0 g/kg). While vehicle injection had no substantial effect on the concentration of 5-HT or DA in either area, injection of ET resulted in dose-related increases in the levels of these neurotransmitters and their metabolites in the LH. ET increased 5-HT and DA release in LH as well as NA, and had no substantial effect on the concentration of 5-HT or DA in either area. ET injection increased SHT- and DA release in LH as well as NA, and suggests that the primary site of action for ET effects on brain monoamines remains to be determined. (Supported by NSF; SBA, and by AFSOR and NIH; BLJ)
469.3  AUTORADIOGRAPHIC COMPARISON OF IMPRIMINE AND CITALOPRAM BINDING IN THE HUMAN HYPOTHALAMUS. K.Y. Little, O.E. Duncan, Dept. of Psychiatry and Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Imipramine binding in post mortem brain tissue has been considered a measure of serotonin uptake sites, and altered binding in suicides suggested as evidence of a serotonergic abnormality. In an initial evaluation of these assumptions, we compared the autoradiographic topography of imipramine binding (Imipramine + [3H]imipramine binding to that of [3H]citalopram binding to Imipramine + [3H]imipramine, a highly selective ligand for the serotonin transporter. Hypothalamic tissue was obtained at autopsy from acute trauma victims with documented suicide attempts, as well as from controls. 

Autoradiograms were prepared using the standard procedure described by the manufacturer (NEN, DuPont). The autoradiograms were digitized and analyzed using a computer-assisted image analysis system. The data were analyzed using a one-way ANOVA with post-hoc analysis. The results showed a significant difference in the density of binding sites between the suicide and control groups. The suicide group had significantly lower binding sites compared to the control group.


NG 108-15 cells are a hybridoma cell line that secretes the neurotoxin 5-HT and is used as a model system for studying the effects of 5-HT on neuronal receptors. In this study, we investigated the presence of 5-HT-paroxetine binding sites in NG 108-15 cells using a radiolabeled ligand. The binding of the ligand was measured using a scintillation counter. The results showed a significant presence of 5-HT-paroxetine binding sites in NG 108-15 cells, which suggests that these cells can be used as a model system for studying the effects of 5-HT on neuronal receptors.
whether this uptake carrier-mediated release is the same for all of these.

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Thus, we determined whether a combination of two fenfluramine (F E N) cause release of serotonin (5-HT) from presynaptic nerve endings. Drug-induced 5-HT release was studied using crude rat brain synaptosomes preloaded with 3H-5-HT (100 nM). First, dose-response curves were determined, with M DM A (40 mg/kg per day, s.c.) or saline. Rats were then collected and processed for 5-HT immunocyto-­ ination. An inhibition of neurotoxicity in forebrain and brainstem. Our results indicate that the ablation of 5-HT axons and terminals produced by MDA is more extensive than previously reported.

These findings suggest that neurotoxic amphetamines administered to young rats may lead to initial regenerative sprouting followed by accelerated aging of 5-HT projections later in life. Thus, despite the early regenerative response of 5-HT neurons after PCA-induced injury, the normal 5-HT innervation pattern is not re-­ established. Supported: NIDA DA-04431 & 271-90-7408.

EFFECTS OF (+) MDMA ON MAO-B ACTIVITY IN RAT BRAIN STEM AND HIPPOCAMPUS. J.T. HOKOTOS LEONARDO and E.C. AZMITIA New York University College of Medicine, NY, New York.

We have previously shown an inhibition of MAO-A activity with an IC-50 of 5x10⁻⁵M in rat brain stem and hippocampus in both aged and immature rats (Azmitia and Azmitia, 1990). We also observed an increase in MAO-A activity at lower concentrations of the drug in two month old rat brain stem and hippocampus. As the primary monoamine oxidase present in serotonergic terminals is MAO-B, we sought to assess the effects of MDMA on MAO-B activity in immature (one month old) rat brain stem and hippocampus as well as mature (two month old) rat brain stem and hippocampus. In brief, rat brain stems and hippocampus were dissected from one month- and two month-old rats and homogenized 10x/v in ice cold 0.01M PB to 10 strokes. The homogenates were centrifuged to obtain a mitochondrial-rich fraction. MDMA was added to homogenates at concentrations ranging from 10⁻⁵M to 10⁻³M in ice cold 0.01M PB to 10 strokes. The homogenates were centrifuged to obtain a mitochondrial-rich fraction. MDMA was added to homogenates at concentrations ranging from 10⁻⁵M to 10⁻³M. MAO-B activity was assayed by measuring 3H-DA degradation product by scintillation counting. An inhibition of MAO-B activity was observed in both age groups with an IC 50 of 5x10⁻⁷M. A stimulation of MAO-B activity was observed in one month old hippocampus in nomanor concentrations (p<0.001). These results may explain some of the actions of MDMA on brain serotonergic function. Supported by NIDA contract 271-87-8143.

SUBSTITUTED AMPHETAMINES CAUSE SEROTONIN RELEASE IN VITRO VIA A COMMON MECHANISM, U.V. Berger, X.F. Gu and E.C. Azmitia, Dept. of Biology, NYU, New York, NY 10003.

The neurotoxic amphetamine derivatives p-chloroamphetamine (PCA), 3,4-­ methyleneoxyamphetamine (MDMA), methamphetamine (MET) and fenfluramine (FEN) cause release of serotonin (5-HT) from presynaptic nerve terminals. Three closely related (independently blocked) by 5-HT uptake inhibitors, suggesting that it proceeds via the uptake carrier. Yet, it is unknown whether this uptake carrier-mediated release is the same for all of these compounds. In our study, we tested the hypothesis that if these drugs had different mechanisms for their effects then their IC₅₀ values would be different when used in combination. Thus, we determined whether a combination of two drugs releases more 5-HT than either drug alone at an equivalent concentration. Drug-induced 5-HT release was studied using crude rat brain synaptosomes preloaded with ¹⁴C-5-HT (100 nM). First, close-dose response curves were determined, then two drugs were combined at their approximate EC₅₀ concentration. EC₅₀ values for the different drugs ranged from 3 uM to 11 μM with PCA > FEN > MET. The release caused by all four drugs was significantly attenuated by the 5-HT uptake blocker fluoxetine (80 nM). In no case did the combinations of two drugs at the EC₅₀ level of releasing cause, then they acted via a common mechanism. Currently, we are using the same experimental approach to determine whether these drugs share a common mechanism when causing toxicity to cultures of serotonergic neurons. Supported by NIDA contract 271-90-7403 and the Swiss National Foundation.


Although MDA is well known to selectively destroy 5-HT axons and terminals in the brainstem, its possible actions on brainstem have not been examined. Therefore, we have compared the effects of MDA on serotonergic projections in both forebrain and brainstem. Animals were injected twice a day, for four consecutive days, with MDA (40 mg/kg per day, s.c.) or saline. Brains were obtained two weeks after the last injection and processed for 5-HT immunochemistry. MDA produced a severe loss of 5-HT immunoreactive fibers and terminals throughout the trigeminal sensory nucleus, vestibular nucleus, and superior olivary nucleus of the brain stem which was comparable to effects in the forebrain structures such as neocortex and hippocampus. Fluoxetine (10 mg/kg, injected l.p., 20 min prior to MDA) blocked all evidence of neurotoxicity in forebrain and brainstem. Our results indicate that the ablation of 5-HT axons and terminals produced by MDA is more extensive than previously reported. (Supported by NIDA Grant DA04944-06.)
CELL BODY LOSS UNDERLIES PERSISTENT SERTONERGIC DEFICITS INDUCED BY (+)-3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN PRIMATE: G. A. Ricarte, L. L. Katz, and G. Hatirasimone* Department of Neurology, Francis Scott Key Med. Ctr., Johns Hopkins School of Medicine and NIDA Addiction Research Center, Baltimore, MD 21224

This study examined the basis for the lasting serotonin (5-HT)-depleting effects of (+)-3,4-methylenedioxymethamphetamine (MDMA) in the primate. Six adult monkeys (Macaca fascicularis) were used. Three animals were administered MDMA (5 mg/kg, s.c., twice daily for 4 days); three saline-treated animals served as controls. Eighteen months later, immunocytochemical studies were performed using an antibody directed at 5-HT. Sections through the forebrain of MDMA-treated animals showed a marked reduction in the number of 5-HT axons in cortical and subcortical regions. Counts of neurons in the dorsolateral, median and B raphe cell groups revealed a concomitant loss of 5-HT-immunoreactive cell bodies. Each of the raphe nuclei examined was comparably affected. These findings indicate that the toxic effects of MDMA in the primate are not limited to axons but involve neuronal perikarya as well. Further, they suggest that cell body loss forms the basis for the persistent 5-HT deficits seen in MDMA-treated animals. Possible permanent effects in humans using MDMA ("Ecstasy") recreationally need to be considered. [USPHS DA05707]

PRENATAL COCAINE AND FENFLURAMINE ADMINISTRATION INHIBIT SEROTONIN AND DOPAMINE FIBER OUTGROWTH.

H. M. Akbari, H. K. Kramer, and E. C. Azmitia

Dept. of Pharmacology, School of Medicine, Washington University, St. Louis, MO 63110.

Prenatal cocaine exposure has been found to disrupt the development of the serotonin (5-HT) and catecholamine systems as assessed by immunocytochemical methods (Akbari et al., 1990). In this study, we examined the effects of prenatal cocaine and fenfluramine exposure on the development of cortical 5-HT and dopamine (DA) innervation.

Cocaine (10 mg/kg, s.c.) or fenfluramine (1 mg/kg, s.c.) was administered to pregnant Sprague-Dawley rats from gestational day 13 to parturition. Cortical 5-HT and DA fiber densities were assessed on postnatal days (PND) 1, 7, and 28 using high affinity (3H) serotonin (5-HT) and dopamine (3H-DOPA) uptake. Prenatal cocaine exposure resulted in a decrease in the 5-HT and DA high affinity uptake on PND 1 and PND 7. On PND 1, forebrain 5-HT uptake levels were decreased to 64% of control and DA uptake was reduced to 63% of control. By PND 28, 5-HT and DA uptake levels were unchanged compared to controls. Likewise, the decline in 5-HT content after MDMA (10 mg/kg) is maximal 2 h after injection in the frontal cortex (73%), the hypothalamus (48%) and the striatum (36%), but is unaffected in the brain stem or the pineal gland. Two weeks after 8 MDMA injections (12 ip, 10 mg/kg/injection) or a single para-chloroamphetamine (pCA, 10 mg/kg) injection, 5-HT levels are significantly reduced (<0.05) in the frontal cortex (38% and 86%), the hypothalamus (21% and 41%) and the striatum (22% and 86%). Brain stem 5-HT content falls 60% after pCA, but is unaffected by MDMA. Pical S-HT content is unaltered by MDMA or pCA administration. These results suggest that the pineal is resistant to the neurotoxic effects of MDMA or pCA. This resistance may be due to different membrane properties of pinealocytes when compared to serotonergic neurons or to the intracellular localization of 5-HT in pinealocytes.

NEUROTRANSMITTERS: MOLECULAR NEUROBIOLOGY

470.1 NEURONAL GABA TRANSPORTER: IN VITRO PHARMACOLOGY AND DISTRIBUTION OF EXPRESSION IN RAT AND HUMAN BRAIN.

Y. Xia, C. W. Montgomery, G. C. Whitty, M. Poosch, G. Kapatos, M. Bannon. Dept. of Psychiatry, College of Medicine, Texas A&M University, College Station, Texas 77843-1114.

The illicit drug, MDMA (ecstasy), produces a sustained depression in serotonin (5-HT) content in many human brain regions of rats. This depression is presumed to be produced by a neurotoxic effect of MDMA at the nerve terminal utilizing a presynaptic 5-HT reuptake system. The presynaptic receptor has large quantities of intracellular cyclic 5HT without nerve terminals or well characterized 5HT reuptake system. By determining MDMA's effects on 5HT content, the mechanism of MDMA's neurotoxicity may be investigated. Male albino rats have been injected with MDMA acutely (p) using several dose and time response protocols or have received MDMA subchronically (sc) to determine if MDMA alters 5HT content. A significant dose dependent decline (p<0.05) in 5HT content 2 h after MDMA administration (10 and 30 mg/kg) is observed in the frontal cortex, the hypothalamus and the striatum, but not in the brain stem or pineal gland. Likewise, the decline in 5HT content after MDMA (10 mg/kg) is maximal 2 h after injection in the frontal cortex (73%), the hypothalamus (48%) and the striatum (36%), but is unaffected in the brain stem or the pineal gland. Two weeks after 8 MDMA injections (12 ip, 10 mg/kg/injection) or a single para-chloroamphetamine (pCA, 10 mg/kg) injection, 5-HT levels are significantly reduced (<0.05) in the frontal cortex (38% and 86%), the hypothalamus (21% and 41%) and the striatum (22% and 86%). Brain stem 5-HT content falls 60% after pCA, but is unaffected by MDMA. Pical S-HT content is unaltered by MDMA or pCA administration. These results suggest that the pineal is resistant to the neurotoxic effects of MDMA or pCA. This resistance may be due to different membrane properties of pinealocytes when compared to serotonergic neurons or to the intracellular localization of 5HT in pinealocytes.

470.2 DIVERSITY OF NEUROTRANSMITTER TRANSPORTER cDNAs IN BRAIN LEBRARIES.


Recent cloning of cDNAs encoding GABA and norepinephrine transporters (GATAB and NET) reveals areas of high sequence homology. This family may include the dopamine transporter that is a major target for cocaine and methamphetamine, as well as the glutamate and serotonin transporters. We have used oligonucleotides corresponding to the 5-H process cDNAs from brain libraries. PCR product TM111size STH amino acid identity with rat GABAB and 54% with NET; this also contrasts with a partial mouse GADABA cDNA which shows 95% identity with the rat sequence. Northern analyses show 4 kb major band that hybridizes with TM111 and is present in mRNA from several brain regions but not from liver, pancreas or eye.

Screening a ventral midbrain library with oligo-nucleotide sequences conserved in GABAB, NET and TM111 reveals 0.005 positives; TM111 sequences are present in 0.001% of plaques from a whole brain library. Neurotransmitter transporter cDNAs are thus individually rare, but members of a family of related genes with sequence conservation in putative transmembrane regions.
470.3 A cDNA ENCODING A NOVEL NEUROTRANSMITTER TRANSPORTER IS SELECTIVELY EXPRESSED IN SUBSTANTIA NIGRA. J. Kits and S. G. Amara. Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510

Degenerate oligonucleotides based on regions of amino acid sequence similarity between the cloned norepinephrine (NE) and GABA transporters were used in a polymerase chain reaction (PCR) with rat midbrain cDNA. The sequence of one of the CDNA amplified in this reaction diverges significantly from the NE and GABA transporter cDNAs, but predicts a protein with extensive amino acid identity with the NE transporter. When used as a hybridization probe this 684 basepair PCR product detects a single 4.4 kilobase mRNA on Northern blots. On blots containing RNA isolated from a variety of specific brain regions, the intensity of this band was strongest in the substantia nigra, with faint signals in the brainstem and olfactory bulb. No hybridizing band could be seen in RNA from cortex, cerebellum, basal ganglia, kidney, lung, adrenal, or PC12 cells. The predicted sequence and tissue distribution of this mRNA identifies this clone as a likely candidate for a dopamine transporter.

470.5 UPTAKE, TRANSPORT AND TRANSLATION OF EXOGENOUS VASOPRESSIN (AVP) mRNA IN BRATTLEBORO RAT HYPOTHALAMIC-NEUROHYPOPHYSIAL SYSTEM


Following our recent detection of oxytocin mRNA in hypothalamic axons by in situ hybridization (Jirikowski et al., 1990) we have extended our studies to AVP mRNA, and have employed Brattleboro rats, genetically incapable of AVP expression, as a means to detect the metabolic fate of exogenous AVP mRNA injected intracerebrally. RNA extracted from hypothalami of osmotically challenged rats as well as synthetic riboprobes coding for the Arg-vasopressin (AVP) precursor were injected into the hypothalmo-neurohypophyseal tract of homozygous Brattleboro rats. A radio-labelled riboprobe coding for AVP was found to be selectively accumulated and transported in axons to magnocellular perikarya within 2 h, while the antisense sequence failed to show cellular accumulation. Brattleboro rats injected with either the natural RNA preparations or with the synthetic sense probe showed immunoreactive AVP in magnocellular neurons and their distal axons by 18 h and a temporary (1-5 days) significant increase in urine osmolarity. Our results suggest that magnocellular hypothalamic neurons are capable of uptake, axonal transport and translation of certain natural and synthetic mRNA sequences. This may reflect a novel means of interneuronal communication generally. Supported by grants AA06420 and NS22347. GFJ is a Heisenberg Fellow.

470.4 ISOLATION OF NEW MEMBERS OF A TRANSPORTER GENE FAMILY BY SEQUENCE HOMOLOGY BASED PCR. J. A. Clark, A. A. Fluet, and S. G. Amara. Department of Molecular Neurobiology, HHMI, Yale University School of Medicine, New Haven, CT 06510.

Comparison of the amino acid sequences of clones encoding a GABA transporter from rat brain and a norepinephrine transporter from human neuroblastoma cells has led to the identification of regions of sequence with significant homology. Degenerate oligonucleotides were designed based on these conserved regions. Using the polymerase chain reaction along with these oligos we have isolated several novel DNA fragments from midbrain, spinal cord, C6 neuroblastoma cell, and retina Y79 cell cDNAs. Sequencing these cloned PCR fragments has revealed a family of genes which are related to both the GABA and norepinephrine clones, but are clearly new members of this sodium-dependent transport family. Northern analysis has shown that each of these potential transporters has its own unique distribution throughout the rat CNS and peripheral tissues. Several of these fragments are presently being cloned in order to obtain full length sequences and allow complete characterization of these transport proteins.

470.6 IMPORTANCE OF POLY-A TAILS FOR EXPRESSION OF EXOGENOUS RNA IN BRATTLEBORO RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM


Poly A(+) and poly A(-) RNA fractions, obtained from the hypothalamo-osmotically challenged Lewis rats as well as cRNA coding for Arg-Vasopressin (AVP) were injected unilaterally into the hypothalmo-neurohypophyseal tract of young homozogous Brattleboro rats. After 2 hours survival, the magnocellular hypothalamic nuclei of animals injected with 1 μg poly A(-) RNA contained Arg-Vasopressin immunostaining and immunoreactive peptide, ipsilateral to the injection site. After 18 hours, the Brattleboro rats injected with poly A(-) contained immunoreactive AVP in the median eminence and in the posterior pituitary lobe. Plasma AVP levels and urine osmolarity of animals injected with poly A(-) and synthetic AVP RNA were significantly increased within 120 minutes and remained elevated for up to 6 days after the injection. Similar effects could be observed with 50 ng of AVP-cRNA. The injection of 1 μg poly A(+) RNA proved to be less effective, despite the presence of much more mRNA coding for AVP. To assess further the importance of the poly A tail in this process, we annealed the poly A(+) fraction with synthetic poly T tails and then treated with RNase H in order to remove partially the poly A tails. The detailed poly A(+) fraction also significantly increased the urine osmolarity of injected Brattleboro rats, suggesting that the absence of poly A tail is of functional importance for the uptake, transport and translation of RNA by magnocellular hypothalamic neurons. Supported by grants AA06420 and NS22347.


We have previously shown that the two GAD proteins, GAD65 and GAD67, are present in cultures of prenatal rat cortex enriched in GABAergic neurons. Total GAD activity was reduced by 80% in cultures and 30% in vivo when intracellular GABA levels were increased >300% by chronic treatment with the GABA-transaminase inhibitor γ-vinylGABA (GVG). Levels of other amino acids (glutamate, glutamine, aspartate and alanine) changed only slightly, in cultures, the effects of GVG were mimicked by adding GABA to the medium, indicating a negative feedback mechanism. Immunoblotting experiments showed that the level of GAD67 protein was reduced by 76% in cultures and 70% in vivo, but the level of GAD65 protein was unchanged by GVG treatment. The possibility that the decrease in GAD67 is due to selective changes in gene expression was investigated. Total RNA and mRNA were extracted from rat brains and cultured neurons. Northern blotting with 32P-labeled cDNA's from the genes coding for GAD65 and GAD67 (Erlander et al., Neuron, in press), showed that the cerebellum and cortex of adult rats and cultured GABAergic cells contain a 3.7 kb GAD67-mRNA. The effect of manipulating GABA levels on the levels and stability of GAD mRNA in cultures and in vivo are under study. Supported by grants MH55664 and NS53102 from USPHS-DHHS.

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471.1


Specific desensitization of activated receptors is mediated through kinase (R) and other cytosolic factors such as guanine nucleotide binding proteins (G, Gi, Go) in the signal transduction cascade. In the retina, S-antigen (SAG) has been identified as an inhibitor of light-stimulated photoisomerization of rhodopsin by R. In neuronal tissues with adrenergic receptors, an analogous protein, beta-arrestin (βAR) has been shown to contribute to homologous desensitization of adrenergic receptors (Lohse et al., 1991, Science 248:1547). Expression of mRNAs and proteins encoding SAG and BAR are detectable in mammalian retina and spinal ganglia (1991, Neuron 5:1461). Antigenic epitopes are shared and highly conserved between members of the arrestin family; however, multiple genes may contribute to different tissue and cell types. Several homologous arrestin-like domains in rod outer segments (OR), and similar proteins have been identified in the brain. Late stage neuronal differentiation of retinoblastoma cells resulted in the production of a protein with similar antigenicity as that seen in rod outer segments. This result suggests that SAG may be a member of a family of proteins involved in receptor desensitization in vivo.

471.2

MECHANISM OF RECEPTOR DYSFUNCTION IN HYPERAERIAL WATERS

J. Young**, T. Annabi, Y. Uchida* and F. Hirata**. "+Dept. of Environmental Health Sciences, The Johns Hopkins Univ., Baltimore, MD 21205 and #Dept. of Pharmacology and Pharmacogenetics, and Institute of Chemical Toxicology, Wayne State Univ., Detroit, MI 48221.

Hyperaerobic environments (Asthma) have been proposed to be a disease of hypermuscarinic and hyper-β-adrenergic dysfunction. In order to study its mechanism, we employed an animal model of guinea pigs sensitized with ovalbumin and subsequently challenged by a single injection of multiple exposures to the antigen. When their tracheal responses to acetylcholine and isoprotorex were measured in vitro, the trachea isolated after multiple challenges were more sensitive to acetylcholine but less sensitive to isoprotorex. However, the binding studies with ([125I]Nal) and [3H]Iscit showed no changes in the number and affinity of M-2-muscarinic and β-2-adrenergic receptors. These observations suggest that immunological reactions occurring in the lungs modify the postreceptor signal transduction steps, possibly G-proteins.

471.3


These results showed that specific lesioning (6-OHDA) or non specific destruction (IC) an increase of α1 adrenoceptor densities (13-43%) was observed at all levels of the spinal cord. Motoneurons are localized presented the highest densities of α1 adrenoceptors, while in the dorsal horn (proximal part) densities were the lowest. This distribution of α1 adrenoceptors along the different segments of spinal cord (cervical, thoracic, lumbar and sacral) of normal rats has been studied by quantitative autoradiography using the specific α1-antagonist [3H]Prazosin as a ligand. The same autoradiography revealed an homogeneous distribution of these receptors along all segments of the spinal cord with a similar pattern in the various subregions of the grey matter at all levels. However, the central area of the grey matter and the region where the motoneurons are localized presented the highest densities of α1 adrenoceptors, while in the dorsal horn (proximal part) densities were the lowest. This distribution of α1 adrenoceptors only partly corresponded to the distribution of NA nerves revealed by immunohistochemistry. 2) After specific lesion of NA system by 6-OHDA IC an increase of α1 adrenoceptor densities (22-42%) was observed at all levels of the spinal cord. 3) Transsection of spinal cord at T8-T9 level induced a significant increase of α1 adrenoceptor densities (22-42%) in downstream segments (lumbar and sacral) as compared to that observed after 6-OHDA lesion. No significant effect has been observed in upstream segments.

471.4


There are numerous reports that chronically administered antidepressants down-regulate beta-adrenoceptor function. We have now compared the triazolobenzodiazepine antidepressant, adinazolam (ADZ), with imipramine (IMI) in this regard. Rats were untreated or received ADZ (0.64 mg/kg/day; 2 or 15 days), IMI (10 mg/kg/day; 2 or 15 days) or saline (15 days). Drugs and saline were administered by osmotic mini-pumps implanted s.c. Hippocampal slices (500 μm) were obtained in the appropriate treatment day for intracellular recording from CA1 neurons. Current injection (0.7-0.9 nA, 100 ms) through the microelectrode elicited 6 to 11 spikes followed by a slow afterhyperpolarization (AHP). In untreated rats, isoprotorex (ISOR) 0.5 μM AHP dose-dependently reduced the AHP by 56% with an EC50 of 80 μM. In untreated rats, the maximum depression was 70% with an EC50 of 20 μM to saline treated rats. Thus, the presence of a mini-pump, perhaps by increasing the level of stress, shifted the ISO dose response curve to the left. In comparison to the saline control, IMI shifted the curve to the right (2 and 15 days) and reduced the maximum inhibition (32%: 15 days). Dose response curves for ISO, in ADZ treated rats (2 and 15 days), were similar to the saline control and each other except for the maximum reduction of the AHP (70% and 58% respectively). In conclusion, implanted mini-pumps up-regulate and IMI down-regulates functional beta-adrenoceptor activity. In contrast, ADZ only marginally affects this.

471.5

Cytoskeletal Aspects of the Regulation of β-ADRENÉRGEIC Receptor mRNA. C. Hough and D.-M. Chiang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

The β1- and β2-adrenergic receptor mRNA levels in C6 glioma cells are differentially regulated in response to isoproterenol. The nature of the changes in β-AR mRNA levels varied with different cell culture conditions. For example, at confluency, β2-AR mRNA is in response to isoproterenol treatment, but at low cell density β2-AR mRNA could be transiently up-regulated. Because the cytoskeleton determines the morphology of cells in culture, we investigated the effect of microtubule-disturbing agents on β-AR mRNA regulation. The microtubule disruptor, colchicine, at 10 μM, induced a time dependent up-regulation of β2-AR mRNA and down-regulation of β1-AR mRNA. Simultaneously, the cells withdrew their processes, leaving the surfaces otherwise confluent sheets of cells. Colchicine did not interfere with isoproterenol induced down-regulation of β2-AR mRNA, but appeared to partially attenuate the down-regulation of the β1-AR mRNA species. Taxol, a microtubule-bundling compound, produced effects opposite to those of colchicine: taxol at 10 μM induced the down-regulation of β2-AR mRNA and early up-regulation of the β1 mRNA species. Taxol, at a higher concentration, produced effects opposite to those of colchicine: taxol at 10 μM induced the down-regulation of β2-AR mRNA and early up-regulation of the β1 mRNA species. These results suggest that aspects of the cytoskeleton, play a role in the regulation of β-AR mRNA.

471.6

EFFECT OF CHRONIC TRICYCLIC ANTIDEPRESSANT TREATMENT ON β1-ADRENERGIC RECEPTOR (β1-AR) mRNA IN RAT FRONTAL CORTEX AND CULTURED GLIOSMA C6 CELLS. K. Hopps, P. H. Fishman and R. S. Duman. Lab. of Molecular Psychiatry, Depts of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06508. Lab. of Molecular and Cellular Neurobiology, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892.

One of the most consistent effects of nearly all classes of antidepressant treatment is the binding site density in rat cerebral cortex. Incubation of C6 glioma cells with a tricyclic antidepressant also decreases β1-AR binding, indicating an effect of the drug treatment direct on receptor coupled signal transduction pathways. The present study examines levels of β1-AR mRNA in rat brain and C6 cells to elucidate the molecular mechanisms mediating the regulation of β1-AR by antidepressant treatments. Absolute levels of β1-AR mRNA were determined by RNA-expression analysis. Riboprobes corresponding to the sense and antisense DNA strand of β1-AR were used with W5 and T4 RNA polymerase. Unlabelled cRNA was used to generate the standard curve. Chronic imipramine administration (21 d, 15 mg/kg, i.p.) significantly decreased levels of β-AR mRNA in rat frontal cortex. The effect was not observed after only 3 or 7 d of treatment. Treatment of C6 glioma cells with desipramine (5 d, 10 μM) also significantly decreased levels of β1-AR mRNA. In contrast, treatment with desipramine did not modify the response to antidepressant treatment may occur through regulation of levels of mRNA and gene expression.
471.7


This study was designed to assess the relative abundance of the mRNAs encoding various forms of 5-HT2 receptors in different brain areas. Twenty-four 90-day-old rats were divided into three control and three drug treated groups. Rats were i. p. injected with 0.75 mg/kg of amphetamine every 2 days and were killed 24 hr after the last injection. The hippocampus (Hp), cerebral cortex (Cortex), and the striatum of rats lesioned with 6-OH dopamine were dissected and membrane fractions obtained for the measurement of 5-HT2 receptors. In the case of alpha-1 receptors, the apparent affinity results from the presence of both hetero and autoreceptors and furthermore it appears to be receptor subtype selective. Since depletion of intracellular ATP with antimycin A has been shown to prevent both internalization and low affinity binding in the case of muscarinic receptors, the effects of antimycin A on both alpha-1 and alpha-2 adrenergic receptors were studied in DDT, MP2 smooth muscle cells, antimycin A pretreatment increased affinity of the agonist epinephrine for beta receptors whereas binding of a non-competitive antagonist was not affected. However, antimycin A did not increase epinephrine affinity for alpha-1 receptors in these cells. In Chinese hamster ovary cells expressing alpha-beta receptors, antimycin A increased affinity of the agonist noradrenaline but not the antagonist metoprolol. Similarly, in Chinese hamster ovary cells expressing alpha-beta receptors, antimycin A increased affinity of the agonist noradrenaline but not the antagonist metoprolol.

471.8

DEAFFERENTATION INDUCES A DIFFERENTIAL PATTERN OF EXPRESSION OF THE VARIOUS GUANINE NUCLEOTIDE BINDING PROTEIN mRNAs IN RAT STRIATUM. M. Memo, A. Valerio*, M. Riboia*, M. Buonamici, P. Sarmientos*. - and P.F. Spano Section of Pharmacology, Dept. of Biomed. Sci. and Biotech., School of Medicine, University of Brescia, Italy.

This research was supported by NIH grant GM36564.

Despite the widespread clinical use of carbamazepine (CBZ), the molecular mechanisms underlying its anticonvulsant and mood-stabilizing effects remain unknown. We have recently demonstrated that chronic in vitro exposure of C6 glioma cells to desipramine (DMI) results in a significant downregulation of ϒ adrenergic receptors (BARs). Given the close structural similarity between DMI and CBZ, and their opposite effects on seizure threshold, we examined the effects of CBZ on BARs in C6 glioma cells and found (p<0.05) in a significant increase in BARs. The upregulation of BARs was evident within 3 days of incubation, and exhibited a dose-response profile (10-100 μM). Moreover, the upregulation of beta receptors was accompanied by a marked increase in ϒ mRNA. CBZ’s effects on ϒ mRNA were also time and dose-dependent (and occurred within the drugs therapeutic range). The mechanism(s) by which CBZ and DMI produce opposite effects on BARs is currently under investigation.


We previously demonstrated that hemisection anterior to the mammillary bodies dramatically elevated expression of galanin in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (Neurosci. 1:115). In order to determine the origin of fibers mediating this effect, we made electrolytic lesions in various posterior hypothalamic sites. We used hybridization histochemistry with 35S-oligonucleotide probes against galanin, as well as cholecystokinin, and angiotensin mRNAs. Other rats' SONs were implanted with micropipettes (Brain Res. 235:174) containing the retrograde fluorescent tracer, Fluorogold. These rats were examined 5 days later for the presence of retrogradely labeled cells in the posterior hypothalamus. Electrocorticostimulation in the region of the paraventricular nucleus and mammillary and premammillary portions of the arcuate nucleus elevated levels of galanin transcripts in the PVN and SON. Lesions of the mammillary and supramammillary nuclei were ineffective. The effective lesions also elevated levels of cholecystokinin mRNAs in the PVN and SON, whereas those for angiotensin were reduced in SON. Fluorogold-retrogradely labeled cells were present in the arcuate and premammillary nuclei after injections that included the SON, although potential contributions from extra-SON uptake are still being analyzed. We are also attempting more specific lesions with ibotenic acid and neonatal monosodium glutamate, but our results presented here suggest that projections from the posterior arcuate/premammillary region modular SON and PVN gene expression.
471.5 THE GABA AGONIST MUSCIMOL REDUCES VASOPRESSIN SYNTHESIS AND RELEASE. Michelle M. Roberts and Alan G. Robinson, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

GABA is a major inhibitory transmitter of the hypothalamo-hypophysial system and GABAAergic mechanisms are believed to modify both basal and stimulated vasopressin release. To study the effect of the GABA agonist muscimol on the synthesis and release of AVP and oxytocin (OT) in the rat, acute intracerebroventricular injection (ICV) of muscimol (160 ng in 1 μl artificial CSF [aCSF]) inhibited the AVP and OT response to hemorrhage and hypertonic saline injection. Following a 5 min hemorrhage, plasma AVP levels were reduced to 26%, 46%, 27% and 59% at 5, 10, 15 and 25 min in muscimol treated rats relative to aCSF. OT was dramatically suppressed to levels 5%, 15% and 24% of control responses. We next demonstrated that chronic infusion of muscimol into the lateral ventricle reduced basal synthesis rates of both AVP and OT. Following a 3 day infusion of 50 ng muscimol per hour, AVP synthesis rates were reduced from a baseline value of 1.5 μg/h to undetectable levels. OT synthesis decreased from a basal rate of 4.1 ng/h to 0.5 ng/h. AVP mRNA levels in muscimol treated rats were reduced to 53% of control. AVP mRNA levels in muscimol treated rats were reduced to 53% of control. Plasma sodium and hydration were normal in all groups of rats. We conclude that acute ICV injection of muscimol inhibits AVP and OT release in response to both hemorrhagic and osmotic stimuli. Chronic infusion of muscimol inhibits basal AVP and OT synthesis and reduces AVP mRNA levels.

These studies indicate that GABA is a major inhibitor of physiologic release and synthesis of AVP.

471.7 ALTERED CNS EXPRESSION OF CYTOPLASMIC AND HETERONUCLEAR VP RNA IN THE BRATTLEBORO RAT. P. Ios, M.D. Brot and D.M. Dorsa, GRECC, Seattle VAAMC, WA 98108 and Dept. of Pharmacology, Univ. Washington, Seattle, WA 98195.

The HOM-BB rat lacks the ability to synthesize VP and its neurohypophysial hormone release is believed to contribute to maintenance of T₄, their activity is believed to contribute to maintenance of T₄ during cold exposure. Because TRH mRNA levels in other hypothalamic nuclei are linked to TRH release (Zoeller et al., Endocrinology 127:2955, 1991), we hypothesized that TRH mRNA levels in POA neurons would be elevated by cold exposure. Using in situ hybridization and single-cell analysis, we found that TRH mRNA levels in neurons of the POA were significantly lower (p<0.01) in animals exposed to 6° C (n=14) compared to 25°C (n=11). Cold exposure produces changes in cardiovascular function which may account for some effects of cold. We manipulated blood pressure with 60 min infusions of Na noradrenaline (SN, n=8), phenylephrine (PE, n=11), or saline (n=14) to initially investigate possible involvement of these TRH neurons in regulating cardiovascular function. Neither drug significantly affected TRH mRNA levels in PVN; nor did SNP affect TRH mRNA levels in POA. However, we did find that SNP significantly elevated TRH mRNA levels in specific raphe nuclei (p<0.01). These data suggest that separate populations of TRH neurons may be involved in different aspects of T₄ regulation and cardiovascular function. (Supported by: AHA, MO Affiliate (RT2) and HL51218 (ICS)).

ENDOCRINE REGULATION II

472.1 INTERACTION OF F8Fa WITH DYNORPHIN IN MODULATING NEUROPEPTIDE SECRETION FROM ISOLATED NERVE ENDINGS OF THE RAT NEURAL LOBE. K. PAYZA, Laboratory of Biochemical Genetics, NIH Neuroscience Center, WAW-113, St. Elizabeth's Hospital, Washington, D.C. 20032.

The neuropeptide FLFPQPRFamide (F8Fa) was tested for modulatory effect on secretion of the hormones oxytocin (OT) and vasopressin (VP) in isolated nerve endings of rat neural lobe, where F8Fa has been localized. The endings were loaded onto 0.45 μm filters, perfused with Ringer containing physiological salts and protease inhibitors, and stimulated by perfusion with 35 mM KCl. The OT and VP secreted into the eluate were measured by RIA. Dynorphin A 1-8 (1 μM) inhibited secretion of both OT and VP. F8Fa (0 μM) blocked the inhibition of OT and VP secretion induced by dynorphin A 1-8. These effects of F8Fa were similar to naloxone (10 μM), which also blocked or attenuated the effect of dynorphin on OT and VP secretion. These results suggest a local modulatory role for F8Fa in the rat neural lobe.

472.2 STIMULATION OF NEUROPHYSIN EXPRESSION IN CULTURED NEURONS BY CYCLIC AMP IS REVERSIBLE. C.D. Stadler and M.J. Gallager,* Univ. Rochester Sch Med, Rochester, N.Y. 14627.

Previous studies have demonstrated that the number of vasopressin (VP) neurons present in primary dopaminergic cultures can be markedly augmented by treatment with drugs that elevate intracellular cyclic AMP (AMP; Goedheer, et al., J Neurosci., 1990). In order to evaluate the effect of this drug treatment on VP secretion by dopaminergic cultures, we have examined primary dispersed dopaminergic cultures derived from 14 day old fetal Sprague-Dawley rats to forskolin (25 μM) and the phosphodiesterase inhibitor, IBMX (50 μM) either continuously or intermittently for up to 12 days. Cultures were collected throughout the culture period for VP radioimmunodassay. At the end of the experiment, cultures were stained immunocytochemically for neurophysin (NP). As reported by previous investigators, exposure to the drugs for 11 days resulted in a 10% increase in the number of NP positive neurons. This increase in the cell number was sustained during longer periods of exposure. During this period the cells continued to increase in size and develop extensive neuritic processes. This increase in cell size and complexity was reflected in an increase in the incorporation of VP in cell protein from 1.4±0.15 pg/ml at 11 days to 8.4±0.6 pg/ml at 32 days of drug treatment. The VP concentration remained undetectable (<1±2 pg/ml) in non-treated cultures throughout this period. The effect on NP expression required the continuous presence of the drugs. Removal of the drugs from day 11-18 of culture resulted in an almost complete loss of NP positive cells, however re-exposure to the drugs restored the NP expression in a time dependent fashion. These results demonstrate that VP secretion and maturation of NP neurons in dispersed dopaminergic cultures can be markedly stimulated by chronic treatment with cAMP elevating drugs, but continuous exposure to the drugs is necessary to sustain the effect. Supported by RO1-DK19761 and PHS 57704703-29.

472.3 OPIOIDS INHIBIT SYNAPTIC INPUTS FROM THE REGION OF THE ORGANUM VASculosum OF THE LAMINA TERMINALS (OVLT) TO THE SUPRAOPTIC NUCLEUS (SON) IN VITRO. S.J.A. MacMillan, Centre for research in Neuroscience, Montreal General Hospital and McGill Univ., Montreal, H3G 1A4.

The activity of SON neurons is thought to be regulated by inputs from the region anterior and ventral to the third ventricle (AV3V). This input which is thought to be important, in part, in the negative control of neurohypophysial hormone release is believed to be opioid sensitive. Using a superfused hypothalamic explant preparation and intracellular recording techniques we have examined the effect of opioids on this input to the SON. Morphine applied to the superfusion line (1-10μM) inhibited the spontaneous firing of MNCs (n=21) but this was associated with only weak membrane hyperpolarizations. However morphine (1μM) profoundly inhibited (30%) spontaneous presynaptic potentials (PSPs). Electrical stimulation of the OVLT region produced short latency short duration mixed excitatory and inhibitory PSPs followed by longer latency excitations in 17 MNCs. Morphine (1μM) inhibited both early and late components of this response (n=11). These inhibitions peaked within 2-5 minutes of morphine reaching the tissue and recovered within 10 minutes of return to control solution and were prevented (n=4) by the opioid antagonist naloxone (1μM). We conclude that opioids exert a presynaptic inhibitory action on inputs from the OVLT region to the SON. (Supported by the MRC.
473.3 OSMOTIC STIMULATION OF THE ORGANUM VASculosum RER TERMINALIS (OVLT) ACTIVATES RAT MAGNOCellular NEUROSECRETORY CELLS (MNCs) IN VITRO. D. Richard and C.W. Bourque. Centre for Research in Neuroendocrinology, McGill University, Montreal, Canada.

The OVLT has been implicated as a central osmoreceptor involved in the regulation of neurohypophyseal hormone release. Possible functional interactions were examined during intracellular recordings obtained in superfused hypothalami from rat hypothalamic explants. Brief (20-50 mOsM) hypertonic stimulation (20-50 mOsM) of the OVLT area induced depolarization and increased firing in each of five local neurons tested. Selective osmotic stimulation of the OVLT also caused increases in the frequency of spontaneous excitatory and inhibitory potentials and accelerated the firing rate in each of 14 MNCs tested. These effects were dose-dependent and persisted for 1-3 minutes before reverting to control rates. In contrast to the pronounced depolarizations (3-10 mV) and decreases in input resistance (10-25%) observed during direct osmotic stimulation of MNCs (n=4), increases in firing rate recorded from MNCs during OVLT stimulation were not accompanied by changes in impedance or membrane potential. These results suggest that neurons located in or near the OVLT convey information concerning fluid osmolality to MNCs via synaptic input to the supraoptic nucleus. Supported by FCAR and the MRC.

473.4 EFFECTS OF NEUROTENSIN ON RAT MAGNOCellular NEUROSECRETORY CELLS (MNCs) IN VITRO. K. Kirkpatrick and C.W. Bourque. Montreal General Hospital and McGill University, Montreal, Canada.

The tridecapeptide neurotensin (NT) has been shown to induce antidiuresis in dogs. In this study we have examined the actions of this peptide on hypothalamic MNCs. Intracellular recordings were obtained from supraoptic nucleus MNCs in superfused rat hypothalamic explants. Stimulation of NT (1-50 nM) induced a long-lasting depolarization and enhanced spike discharge in each of 23 cells tested. Eight of these cells were classified as being vasopressinergic on the basis of their ability to display phasic firing. These effects were mimicked by the NT B-3 fragment (n=7) and in Ca++-free solutions containing Mn++ (2mM, n=3). In addition to these NT decreased the magnitude of the Ca++-dependent afterhyperpolarization (AHP) that follows spike trains in MNCs (n=4), as well as the corresponding outward current measured under hyperpolarizing clamp (n=2). Intracellular injections of Cs++ sufficient to abolish the AHP (n=4) did not prevent NT-induced depolarizations. These results suggest that activation of postsynaptic NT receptors can contribute to the regulation of vasopressin release by exciting MNCs in two distinct manners. Supported by FCAR and MRC.

473.5 VASOPRESSIN RELEASE BY CENTRAL ANGIOTENSIN II IS MEDIATED THROUGH AN ANGIOTENSIN TYPE-1 RECEPTOR AND THE DRINKING RESPONSE IS MEDIATED BY BOTH AT-1 AND AT-2 RECEPTORS. D. Phillips, Dept. of Physiology, College of Medicine, University of Florida, Gainesville, FL 32610.

Angiotensin II, given centrally, releases vasopressin, increases blood pressure and stimulates drinking. With the advent of new receptor subtype antagonists, we are able to investigate whether the effects are mediated by AT-1 receptors or AT-2 receptors in the brain. Adult male Sprague Dawley rats were cannulated and 5 days later catheterized in the common carotid artery for blood pressure measurements. All experiments were carried out in chronic rats. Three treatments were given i.v.t. in 2 μl ACSF at 30 min intervals. 1) 50 ng Ang II, 2) AT-1 antagonist 0.7 μg DuP or AT-2 antagonist 7.6 μg EXP, 3) 50 ng Ang II to test for recovery. Controls to test repeated doses were also used. Blood pressure and drinking responses were recorded and an arterial blood sample drawn for radioimmunoassay of arginine vasopressin.

The results showed that plasma AVP release in response to Ang II, is reduced 70% by AT-1 antagonism but not altered by AT-2 antagonists. EXP had no significant effect on the response, but DuP significantly blocked the pressor response (p<0.01). Drinking was also antagonized by DuP (64%), and partially by EXP (50%). The results show that the central vasopressin and blood pressure responses to Ang II i.v.t. are mediated by AT-1 receptors and the drinking response is mediated by AT-1 and partly by AT-2 receptors.

473.6 A D1-DOPAMINE RECEPTOR AGONIST AND ANGIOTENSIN II STIMULATE OCTYTOCIN RELEASE IN THE LACTATING RAT VIA ACTIONS IN MAGNOCellular HYPOThALAMIC REGIONS. B.L. Parker and W.R. Henderson. Dep. of Pharmacology, University of Tennessee-Memphis, Memphis, TN 38163.

Dopamine activates OT release in the lactating rat, and our recent results suggest that the D1 receptor is preferentially involved (Neuroendocrinology 53: 493, 1991). The objective of the present studies was to test whether D1 stimulation of OT release occurs via an action in the hypothalamic magnocellular nucleus. Lactating Holtzman rats received cannula implants in the third ventricle (3v) or unilaterally above the paraventricular nucleus (PVN) or supraoptic nucleus (SON) at 4-7 days postpartum. The D1 agonist SKF 38393 (SKF) was injected into the 3v at 12.5 or 50 μg/μl or into the PVN or SON regions at 8 μg/μl. Controls received an equal volume of vehicle. All sites were subsequently treated with angiotensin II (AI, 95 pmoles) as positive control. SKF dose-dependently elevated plasma OT at 5-60 min following injections into the 3v. Highly significant stimulation was also seen following injection into the SON, and a lesser, but significant, stimulation was apparent after treatment of the PVN. The increase in plasma OT after 50 μg SKF in the 3v was attenuated by prior iv injection of the D1 antagonist SC35 23390 (750 μg/kg). All significantly stimulated OT release from all three injection sites, but the same dose was ineffective after iv administration. Plasma OT levels were unaffected by vehicle injection at any site. SKF in the 3v also dose-dependently increased plasma concentrations of prolactin (PRL) and produced a strong priming of PRL release to subsequent 3v injection of AI.

473.7 OSMOSENSITIVITY OF MAGNOCellular NEUROSECRETORY CELLS (MNCs) ISOLATED FROM THE SUPRAOPTIC NUCLEUS OF THE ADULT RAT. Stephane H.R. Oliet and Charles W. Bourgue. Centre for Research in Neuroendocrinology, Montreal General Hospital and McGill University, Montreal, PQ.

Small (1-5%) increases in plasma osmolarity induce the release of oxytocin and vasopressin from the axon terminals of MNCs in the neurohypophysis. While a number of other osmoresponsive inputs may be involved, the synaptic input from the lamina terminalis (OVLT) appears to be critical. The OVLT has been implicated as a central osmoreceptor involved in the regulation of neurohypophyseal hormone release. Possible functional interactions were examined during intracellular recordings obtained in superfused hypothalami from rat hypothalamic explants. Brief (20-50 mOsM) hypertonic stimulation (20-50 mOsM) of the OVLT area induced depolarization and increased firing in each of five local neurons tested. Selective osmotic stimulation of the OVLT also caused increases in the frequency of spontaneous excitatory and inhibitory potentials and accelerated the firing rate in each of 14 MNCs tested. These effects were dose-dependent and persisted for 1-3 minutes before reverting to control rates. In contrast to the pronounced depolarizations (3-10 mV) and decreases in input resistance (10-25%) observed during direct osmotic stimulation of MNCs (n=4), increases in firing rate recorded from MNCs during OVLT stimulation were not accompanied by changes in impedance or membrane potential. These results suggest that neurons located in or near the OVLT convey information concerning fluid osmolality to MNCs via synaptic input to the supraoptic nucleus. Supported by FCAR and the MRC.

473.8 DISTRIBUTION AND ORIGINS OF GABAergic PROJECTIONS TO THE PARAVentricular NUCleus. B.I. Roland, M.R. Brown and P.E. Samuels. The Salk Institute, La Jolla, CA 92037 and UCSD Medical Center, San Diego, CA 92103.

Axonal transport and immunohistochemical methods were used to characterize the organization of glutamic acid decarboxylase-immunoreactive (GAD-IR) projections to the paraventricular nucleus of the hypothalamus (PVH) in the rat. In line with prior reports, GAD-IR varicosities were found to be densely and quite uniformly distributed throughout the hypothalamus, including the PVH and the supraoptic nucleus (SO). Small crystalline implants of the retrograde tracer, true blue, into the PVH labeled GAD-IR cells in the anterior perifornical region, portions of the anterior hypothalamus immediately ventral to the PVH and just dorsal to the optic tract at the level of the SO, and within the PVH itself. Because possible uptake of retrograde tracer by local dendritic processes might have yielded false positive results, combined astroglial transport (PFA-1)-immunohistochemical approach was used in an attempt to confirm some putative local sources of GAD-IR inputs. Tracer injections in the region immediately ventral to the PVH, or within the anterior third of the nucleus, labeled moderate axonal projections to the PVH; a variable, and generally small, proportion of anterogradely labeled axons and terminals in the PVH also displayed GAD-IR. Some PFA-1-L injections also labeled GAD-IR projections to the STh. These results suggest that GABAergic projections to the various vesciculometer cell types in the PVH and SO arise at least principally from several diffusely distributed local sources. Pro- and intra-nuclear GABAergic neurons could provide an intermediary by which documented (and generally inhibitory) lamina system influences on neuroendocrine function are exerted.

Oxytocin (OT)- and vasopressin (VP)-secreting neurons exhibit different patterns of spontaneous and evoked activity in vivo. To determine whether differences exist in the membrane properties of these two cell types, supraoptic neurons were recorded intracellularly from rat supraoptic nucleus. Neurons with bicuculline or NMDA but not neuropeptide Y and immunocytochemically identified as either OT or VP-containing. OT and VP neurons exhibited similar UV relationships, input resistances, and membrane time constants. However, positive ramping responses, brief spike trains, both types exhibited brief afterhyperpolarizations, although the amount was variable across neurons. Both types exhibited a similar long lasting afterhyperpolarization following a spike train which was reduced by strychnine (up to 100 μM), known to block a Ca²⁺-dependent K⁺ current in these neurons. The great majority of VP neurons exhibited a depolarizing afterpotential (DAP) following brief spike trains. When a step current injection which altered the membrane potential failed to force VP neurons to exhibit phasic bursting activity, including those few with a DAP. Many VP neurons adopted or spontaneously exhibited the phasic pattern, but several did not, and instead fired continuously, not unlike OT neurons.

The results on phasic bursting are consistent with those from other labs, i.e., neurons exhibiting this pattern contain VP. However, the lack of this activity, or the expression of continuous firing, is clearly not a unique signature for OT neurons. Similarly, while the voltage-sensitive DAP is undoubtedly related to the phasic bursting pattern, while characteristic of, is not unique to VP neurons. Supported by NIH NS23941(W.EA) and NIH NS19933 (BNS).

473.11 BICYTIC ACID LESIONS OF MEDIAN PREOPTIC NUCLEUS DO NOT INFLUENCE BARORECEPTOR INDUCED INHIBITION OF SUPRAOPTIC VASOPRESSIN-SECRETING NEURONS IN THE RAT. R. Nissen, B. Nisen & L.P. Renaud. Neurosciences Unit, Ottawa Civic Hospital, Ottawa, Ontario Canada K1Y 4G9.

Electrical stimulation of two forebrain structures, the diagonal band of Broca (DBB) and the median preoptic nucleus (MPO), induces GABA, receptor mediated sympatho-inhibition of hypothalamic vasopressin (VP) secreting neurons. The DBB relays inhibitory baroreceptor information associated with increases in blood pressure to VP secreting neurons. The purpose of this study was to determine whether MPO neurons are similarly involved in baroreceptor-induced suppression of supraventricular VP neurons. Male Long-Evans rats were anesthetized with ketamine and stereotaxically injected with 0.25μl of bicytic acid (25μg) into the DBB or MPO. Following a minimum of 3 days recovery, animals were reanesthetized with ketamine and prepared for extracellular recording from identified SON neurons using a transparyngial approach. The spontaneous activity of the VP neurons tested in control, DBB lesioned or MPO lesioned rats were not different (8.5±.7, 7.0±.6, 9.9±.8 spikes/sec respectively). While acute increases in blood pressure, achieved by intravenous administration of noradrenalin (10μg/kg), reliably arrested the activity of 18/18 neurons in the control group, 20/20 lesioned rats did not exhibit a DA in a majority of cases. Continuous current injection into the median preoptic neurons, which are involved in body fluid homeostasis, are not necessary for the transmission of inhibitory baroreceptor information to the supraventricular VP-secreting neurons. (Supported by FRSQ, Ontario Heart & Stroke, MRC & NIMH of Australia)

473.13 SODIUM DEPRIVATION BLUNTS HYPOVOLEMIA-INDUCED NEUROHYPOPHYSIAL SECRETION OF OXYTOCIN (OT) AND VASOPRESSIN (AVP) IN RATS. E.M. Stricker & J.O. Verberls. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, PA 15260.

The present study determined the effects of dietary sodium intake on neurophysiologically identified OT and AVP secreting neurons in response to plasma volume deficits in rats. Adult male rats were fed either standard sodium-rich diet or isosodium-deficient (NaD) diet for 8 days, and then injected with 5-10 ml of 30% polyethylene glycol (PEG) solution to induce hypovolemia. Neurons were maintained on either diet for 7 days. A control diet of OT did not begin until plasma volume deficits reached 25-30%, while AVP secretions increased gradually over this range. However, when hypovolemia was more pronounced, secretion of both hormones was significantly blunted in rats given NaD diet. These effects did not reflect a reduced responsiveness of the neurohypophyseal system because normal secretion of AVP and OT was observed after iv infusion of 2 NaCl. Nor did they reflect insensitivity to sodium because rats allowed access to water drank normally after 30% PEG treatment (15.3±0.7 ml in 7 hr). Instead, the results likely represent a specific reduction of the stimulatory baroreceptor inputs into OT and AVP neurons during dietary sodium deprivation. In rats maintained on high sodium NaD diet for 8 days, and the known co-activation of magnocellular and parvocellular OT neurons under some circumstances, the present observations are consistent with recent findings relating activity in central OT neurons with inhibition of salt appetite in rats.

473.14 CENTRAL SOMATOSTATIN INHIBITS CHOLECYSTOKININ-STIMULATED RELEASE OF MAGNOCELLULAR OXYTOCIN IN CONSCIOUS RATS. R.E. Blackburn, E.M. Stricker, and J.O. Verberls. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, PA 15261.

Recent evidence has demonstrated a direct projection from the nucleus tractus solitarius (NTS) to neurohypophyseal (OT) cells in the hypothalamic supraoptic nucleus (SS-28). Since NTS afferents are known to influence release of OT in response to peripheral cholecystokinin (CCK) injections and since SS-28 has been shown to suppress activity of SON cells in vitro, we studied the effect of central SS-28 administration on CCK-stimulated OT release. Conscious male rats were given either SS-28 (300 ng/rat in 5 μl) or vehicle (aCSF, 5 μl). Blood samples for basal OT levels were obtained 30 min later, or rats were injected with CCK (10 μg/kg or 100 μg/kg) and then sampled 5 min later. In another group, plasma OT levels were measured 30 min after administration of hypertonic saline (5% NaCl). Plasma OT levels (pg/ml) in each group are summarized below (** p < 0.01 compared to aCSF-treated control rats).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SS-28 (pg/ml)</th>
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<tbody>
<tr>
<td>Basal</td>
<td></td>
</tr>
<tr>
<td>CCK</td>
<td></td>
</tr>
<tr>
<td>10 μg/kg</td>
<td>35.2 ± 2.4</td>
</tr>
<tr>
<td>100 μg/kg</td>
<td>42.2 ± 17.8</td>
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These data indicate that the administration of SS-28 significantly inhibits CCK-induced OT release, while leaving osmotically stimulated OT release intact. Our results therefore provide one of the first demonstrations of the importance of specific cell-to-cell-chemotaxing NTS pathways on neurohypophyseal secretion in conscious rats.
NALOXONE INHIBITS SODIUM APPETITE IN THE HYPOVOLICEMIC RAT BY A MECHANISM INVOLVING CENTRAL OXYTOCIN. J.G. Verbalis, R.E. Blackburn and E.M. Baertschi. Departments of Anatomy & Neurobiology and Behavioral Neurosciences, University of Pittsburgh, Pittsburgh, PA 15261.

Sodium appetite in the rat is commonly associated with suppressed plasma oxytocin (OT) levels but can be inhibited by a variety of treatments that lead to increased pituitary OT secretion. Systemic administration of OT does not, however, abolish saline ingestion, and this has led us to postulate that it is central rather than the peripheral OT which mediates the hypovolemic response. To test our hypothesis, we investigated the effect of peripheral administration of naloxone, a treatment known to simultaneously both central and peripheral OT secretion, on saline ingestion in the polyethylene glycol (PEG)-induced hypovolemia model of sodium appetite. Male rats were treated with PEG (30% w/w, 5 ml/kg, sc) and deprived of food and water for 24 h, after which they were injected with either naloxone (2.5 mg/kg in 2 ml/kg) or isotonic saline (2 ml/kg) 30 min prior to a 5-h drinking test period. Naloxone administration was associated with a marked increase in plasma OT levels (252.0 ± 45.4 pg/ml; n = 10) compared to basal saline-treated rats (n = 6, p < 0.001). In one-bottle (0.5 M NaCl) drinking tests naloxone completely blocked the sodium ingestion observed in control rats (5-h intake = 8.3 ± 0.9 ml). This inhibition of 0.5 M NaCl ingestion by naloxone could be reversed by prior intravenous pretreatment with 0.01 mg/kg of the selective 5-HT2 receptor antagonist LY 53857 (100 μg/kg, iv) at doses of either 10 μg (5-h intake = 5.9 ± 1.4 ml) or 50 μg (5-h intake = 4.6 ± 0.7 ml). These data demonstrate that naloxone, like other stimuli for OT release, completely inhibits the saline drinking response. Since inhibition could be reversed by iv pretreatment with an OT receptor antagonist, the results also provide evidence that brain OT participates in naloxone-mediated inhibition of hypovolemia-induced sodium appetite in rats.

CENTRAL CATECHOLAMINERGIC PATHWAYS ARE REQUIRED FOR VASOPRESSIN RELEASE FOLLOWING SPLANCHIC OSMORECEPTOR ACTIVATION. M.S. King and A.J. Baertschi. Neuroscience Program, University of Virginia, Charlottesville, VA 22908.

Previous studies in conscious rats demonstrated that peripheral osmoreceptors located at postganglionic sites within the central mesencephalon are activated following hypotonic gastric infusions and project to the CNS through a spinal, and not a vagal, afferent pathway (Cho-Kwon and Baertschi, AJP, '91). In the current set of experiments, to determine the role of cell bodies and fibers of passage in this area, chemical lesions were induced one week prior to gastric infusions with 6-OHDA (50 μg/kg, 2 ml/kg). Microinjections of 5-HT (6-OHDA; 4 μg/kg/2 μl in 0.1% ascorbic acid) evoked vasopressin levels at doses of either 10 μg (5-h intake = 5.9 ± 1.4 ml) or 50 μg (5-h intake = 4.6 ± 0.7 ml). These data demonstrate that naloxone, like other stimuli for OT release, completely inhibits the saline drinking response. Since inhibition could be reversed by iv pretreatment with an OT receptor antagonist, the results also provide evidence that brain OT participates in naloxone-mediated inhibition of hypovolemia-induced sodium appetite in rats.

FLUOXETINE INDUCES VASOPRESSIN ABNORMALITIES IN ACTIVITY-BASED ANOREXIA. P.F. Araújo, T.S. Beg, J. Ahniit, S. Dowling and T.J. Laurson. Dept. Anatomy & Neurobiology & Internal Medicine, Section Virginia Medical School, Norfolk, VA 23501; V.A. Med. Ctr., Hampton, VA 23667.

Vasopressin (VP) and serotonin (5HT) abnormalities occur in anorexia nervosa (AN). It has been proposed that fluoxetine (FLX), an indirect acting 5HT agonist, may be useful in the treatment of AN. Because of the relationship between exercise and AN, we have been exploring activity-based anorexia (ABA) in the rat (restricted feeding 15 hr/day, free running wheel access 22.5 hrs/day). This experiment determined the effects of FLX on VP systems were evaluated. Adolescent male rats (N=83) were divided into 3 subgroups (N=28 each) following a 25% weight loss: ABA (n=28), saline (n=28), and control groups, which included weight-matched animals. ABA rats were treated with FLX (15 mg/kg, ip) or vehicle under resting, saline, and E.M. Strieker. Departments of Medicine and Behavioral Neuroscience, University of Kansas Medical Center, Kansas City, KS 66103 and University of Pittsburgh, Pittsburgh, PA 15260.

We have demonstrated that intraventricular (iv) serotonin (5-HT) increases blood pressure and decreases heart rate. FLX, a selective 5-HT2 receptor antagonist, was used to determine if 5-HT acts at the 5-HT2 receptor to increase plasma vasopressin (AVP) levels. FLX (2.5 μg in 5 μl saline containing 0.1% ascorbic acid) was administered iv to conscious male Sprague-Dawley rats instrumented with venous and arterial catheters. Arterial blood samples (0.5 ml, replaced with sterile saline) were obtained prior to and 5, 15 and 30 minutes after the injection of FLX. In rats pretreated with saline iv, 5-HT increased plasma AVP at 5, but not 15 or 30 minutes, the iv injection of saline did not alter AVP. In rats pretreated with the selective 5-HT2 receptors antagonist LSD 5387 (100 μg/kg, iv), 5-HT was no longer capable of increasing AVP. LV 5387 alone did not alter plasma in addition, prolactin and corticosterone were measured in these same rats. As anticipated, 5-HT increased plasma levels of both hormones. In contrast, plasma renin activity was not affected by 5-HT. The 5-HT-induced increase in prolactin and corticosterone was not altered by the 5-HT2 receptor antagonists. These data suggest: 1) 5-HT increases AVP secretion by activation of 5HT1 receptors. Plots of time-course similar to that observed previously in our hemodynamic studies, and 2) although 5-HT also was capable of increasing the secretion of prolactin and corticosterone, these effects are not mediated exclusively by activation the same 5-HT1 receptor subtype. [Supported by a Grant-in-Aid from the Kansas Heart Association to RHA]
473.21
THE EFFECT OF SALT LOADING ON SEROTONERGIC ACTIVITY IN SPECIFIC BRAIN REGIONS OF THE RAT. J.A. Sadyoff and M.S. Brownfield. Department of Neurology and Psychiatry, School of Medicine, University of Washington, Seattle, WA 98195.

The aim of this study was to establish potential brain sites where serotonin (5-HT) influences osmoregulation of water and salt. We observed that depletion of brain 5-HT with 5,7-dihydroxytryptamine blocks the increase in plasma vasopressin due to i.p. hypertonic saline administration. Groups of 10 male rats were given water (0%) or 2% NaCl (SL) to drink for 2 days and killed by decapitation. The midbrain (MB), hippocampal (H), basal hypothalamus (BHT), dorsolateral hypothalamus (DHT), ventral forebrain (VF), septum (SE), and caudate nucleus (CN) were dissected. High pressure liquid chromatography with electrochemical detection was used to measure 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and dihydrophenylacetic acid (DOPAC). 5-HIAA and DOPAC are the major metabolites of 5-HT and dopamine respectively. Both 5-HIAA/5-HT ratio was used as one of the indexes of serotonergic activity.

Analysis of covariance by brain region plotting 5-HIAA vs 5-HT revealed a similar pattern for the MB, BHT, and VF regions. This striking pattern for the MB, BHT, and VF indicated that salt loading increased both 5-HT and 5-HIAA. The SG 5-HIAA/5-HT ratio was increased compared to the W group in the MB (p <.01) and CN (p <.05). The SL group 5-HIAA was increased compared to the W group in the BHT (p <.01) and H (p <.05). Salt loading decreased 5-HT in the CN (p <.05). There were no significant changes in DOPAC concentration due to salt loading suggesting dopamine is not involved in the osmoregulatory response to salt loading.

These results demonstrate salt loading increases serotonergic activity in the brain regions that previous lesion studies have proven to be essential for osmoregulation of vasopressin. This study suggests a serotonergic pathway linking the MB to the BHT and VF is the circuit for osmoregulation of vasopressin by 5-HT. Supported by Univ. Wisc. Grad. Sch.

473.22

Recent studies (Meeker et al. 1989, 1991; Swanson et al. 1991; Van den Pol et al. 1990) suggest that glutamate plays an important role in the excitatory control of vasopressinergic magnocellular neuroendocrine cells. To assess the influence of glutamate on vasopressin secretion we stimulated acute hypothalamo-neurohypophysial explants from male Sprague-Dawley rats in vitro with 10^-5 M glutamate by perfusion or by direct microinjection into the supraoptic nucleus. Glutamate administered by microinjection or by perfusion induced a significant and rapid release of vasopressin not observed in hypothalamo-neurohypophysial explants from controls or in hypothalamo-neurohypophysial explants perfused or microinjected with other putative neurotransmitters. These results indicate that glutamate is a potent stimulus for vasopressin release even when locally applied to the supraoptic nucleus. The ability of glutamate to induce vasopressin release from the hypothalamo-neurohypophysial explant is consistent with its proposed role as an important excitatory neurotransmitter in the vasopressinergic magnocellular neuroendocrine system. Supported by NIH Javits Award NS 13411.

ENDOCRINE REGULATION III

474.1

The intermediate lobe of the rat pituitary consists of a heterogeneous population of endocrine cells synthesizing α-MSH and β-endorphin. The intermediate lobe cells secrete these hormones under neural regulation from several loci. Several neurotransmitter-specific systems are involved in this regulation. However, it is not known whether this system affects a particular subpopulation of cells or whether all cells receive multiple innervation. We have used immunohistochemical techniques to visualize serotonin-, dopamine-, GABA-, enkephalin- and acetylcholine-containing systems and computerized image analysis to study the distribution of these innervation systems within the lobe. Co-localization of these substances was also examined. Our results favor the notion that most if not all intermediate lobe cells receive innervation from serotonin-, dopamine- and GABA-containing systems. This makes the intermediate lobe a useful model to study the mechanisms of multitransmitter regulation by converging innervation.
474.3

CHARACTERIZATION OF GLUTAMATE RECEPTORS IN THE NEUROENDOCRINE HYPOTHALAMUS. B.B. Meker, R.S. Greenwood and J.N. Hopkins. Department of Neurology and Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

NMDA and non-NMDA glutamate receptors were identified in the rostral hypothalamic nucleus paraventricularis (nucleus of the rostrum) using [3H]glutamate binding to washable membranes. Glutamate (1 mM) and 2-amino-5-phosphonovaleric acid (APV; 0.2 mM) were used to define specific total binding and non-specific binding, respectively. Non-NMDA type receptors greatly exceeded the number of NMDA receptors in the hypothalamus by a factor of approximately 10-fold. Saturation binding data revealed the existence of a high affinity component with a KD of 0.2-4 x 10^(-7) M and a Bmax of 130-150 pmol/mg issue tissue. NMDA binding sites were barely detectable with an approximate KD of 0.2 x 10^(-7) M and a Bmax of 1-2 pmol/mg issue tissue. The high- and low-affinity glutamate subtypes contrasted with the hippocampus from the same brain where similar levels of both NMDA and non-NMDA glutamate receptors were observed. Water deprivation for 48 hrs resulted in an increase in the number of non-NMDA receptors in the hypothalamus with no change in the KD. Thus, the rostral hypothalamus appears to contain mostly non-NMDA receptors. A functional link between these receptors and the various neuroendocrine system which regulates water balance is suggested by the increase in receptors in response to water deprivation.

Supported by NIH Grant W3 1411.

474.4

METABOTROPIC GLUTAMATE RECEPTORS ARE EXPRESSED IN PRIMARY CULTURES OF HYPOTHALAMIC NEURONS. M.A. Sorino, G. Alego*, F. Nicotelli and P.L. Caroni. Department of Pharmacology, University of Pavia, School of Medicine, Catania, and *Chair of Pharmacology, University of Pavia, School of Dentistry, Pavia, Italy.

Glutamate has been shown to play an important role in neuroendocrine regulation in the hypothalamus. The control of neuroendocrine secretion by glutamate has been related to metabotropic glutamate receptor, a group of receptors that are linked to the opening of ion channels. Thus, quisqualate, kainate and N-methyl-D-aspartate (NMDA) have been found to stimulate luteinizing hormone-releasing hormone (LHRH) and vasopressin release in the hypothalamus in vivo. We have previously shown that metabotropic glutamate receptors, which are thought to result in the stimulation of phosphoinositide (PI) hydrolysis, are present at the hypothalamus, and that they have a typical developmental profile that may suggest a role for these receptors in the maturation of hypothalamic neurons. The present paper used immunoprecipitated PI hydrolysates induced by glutamate in primary cultures of hypothalamic neurons. Glutamate, as well as quisqualate, a metabotropic receptor agonist, produced a concentration-dependent stimulation of PI turnover (as assessed by measuring [3H]inositol monophosphate production) that appeared after 7 days of maturation in vitro and was still present at 14 DIV. Lactotropin receptor agonist such as kainate, NMDA and -amino-3-hydroxy-5-methyl-4-isoxazolpropionic (AMPA) were inactive. At 14 DIV, 100 μM glutamate induced a stimulation of 169 ± 16% of control whereas 100 μM norepinephrine activated PI turnover by 23 ± 2%. Simultaneous addition of glutamate and norepinephrine resulted in an additive effect. The presence of glutamate metabotropic receptors in primary cultures of hypothalamic neurons, with the lack of data regarding an involvement of this receptor in the control of neuroendocrine secretion, suggest a role for the metabotropic glutamate receptor in the maturation of hypothalamic neurons as already shown in other brain areas.

474.5


The hypothalamic paraventricular nucleus (PVN) plays a major role in the hypothalamic-pituitary system. We have previously demonstrated that the incubation temperature may alter the hypothalamic-pituitary system. We investigated the role of hypothalamic PVN in the response to cold exposure. The hypothalamic PVN of Sprague-Dawley rats were harvested and either incubated at 37° C or cultured at 4°C. Blood samples were obtained at different time points after cold exposure. The data showed that the incubation temperature significantly affected the plasma levels of ACTH, TSH and glucocorticoids. The results suggest that the hypothalamic PVN may be involved in the regulation of the hypothalamic-pituitary system.

474.6

ALPHA-2 ADRENERGIC INPUT IN LOCUS COERULEUS MODULATES THE RELEASE OF ADRENOCORTICOTROPIN IN CATS. D. Carlson and D. Zhang, Deps. of Surgery and Physiology, Univ. of Maryland Sch. Med., Baltimore, MD 21201.

Previous evidence suggested that noradrenergic input in the ventroventral locus coeruleus (vLC) increased in response to hemorrhage. To investigate the possible role of this response in the control of ACTH release, microinjections (100 nl/min for 2 min) of several agents were made at 42 sites in 21 cats anesthetized with chloralose. All sites were located in the lateral hypothalamus (LH) or the n. paraventricularis (PVN) or the n. supraopticus (SON) or the vLC. Microinjections of adrenaline (10 μM, GLU) and vehicle (VEH), 38 were tested with the α2 agonist, clonidine (1 μM, CLON). Plasma ACTH was measured by radioimmunoassay. Results were tested statistically by repeated measures ANOVA. GLU at 9 sites in the region of the vLC elicited a significant increase in ACTH at 4 and 6 min (P<0.01) from the onset of injection at 4 min. Responses were tested statistically by repeated measures ANOVA. GLU at 8 sites in this region did not change in ACTH. Both of these latter responses differed from that to GLU (P<0.01). Injection of CLON at 6 sites in the vLC elicited a significant decrease in ACTH at 4 and 6 min (P<0.05) from the onset of injection at 6 min. Injections of all agents at sites remote from the region of the vLC elicited no change in ACTH. In an increase in noradrenergic input in the vLC may increase the release of ACTH. This may serve to modulate the influence of the vLC on ACTH release in response to cold exposure.

CRF ANTAGONIST CRF 9-41. W. Rowe, V. Viau, M.J. Meaney, R. Ouirion, with extensive projections throughout the brain. This suggests that time period. Basal secretion of MCH after 5 days of culture was to establish that the peptide is secreted by mammalian cells, we have examined the regulation of MCH secretion from primary hypothalamic cultures of 7 day old rats, employing a sensitive radioimmunoassay for MCH. Secretion was measured over a 6 hour time period. Basal secretion of MCH after 5 days of culture was 54±8 pg/dish/6h, 98±16 pg/dish/6h after 8 days, and increased to 190±11 pg/dish/6h after 10 days in culture and remained at this level for up to 2-3 weeks. On day 8 of culture, the cyclic AMP analogue, 8-Br-cAMP (10mM) increased secretion to 573% of this level for up to 2-3 weeks. On day 8 of culture, the cyclic AMP stimulator, forskolin (10μM), increased secretion to 140%. The cAMP analogue, 8-Br-cAMP (10μM) increased secretion to 573% of control, and B-Br-cyclic GMP increased secretion to 261%. The forskolin ester, TPA (10μM), increased secretion to 133%. Cell content of MCH on days 8, 10, and 12 of culture was 2±1.2, 9±14, 47±12, and 48±6 pg/dish respectively, and this was not significantly changed by any treatment. These results suggest that hypothalamic cells in culture indeed produce MCH, and that this secretion may be regulated by multiple second messenger systems.


The role of neurotensin (NT) as a neuromodulator in the CNS has focused mainly on the mesocorticolimbic dopaminergic system where NT receptors are localized on the majority of DA neurons. However, NT may also serve a neuroendocrine role as a modulator of HPA activity. Intraventricular (iv) administration of NT has been previously reported to increase circulating ACTH and corticosterone (B) levels (Gudelsky et al., Neuroendocrinology, 1989). We investigated the regulation of MCF secretion from primary hypothalamic cultures of 7 day old rats, employing a sensitive radioimmunoassay for MCH. Secretion was measured over a 6 hour time period. Basal secretion of MCH after 5 days of culture was 54±8 pg/dish/6h, 98±16 pg/dish/6h after 8 days, and increased to 190±11 pg/dish/6h after 10 days in culture and remained at this level for up to 2-3 weeks. On day 8 of culture, the cyclic AMP analogue, 8-Br-cAMP (10mM) increased secretion to 573% of control, and B-Br-cyclic GMP increased secretion to 261%. The forskolin ester, TPA (10μM), increased secretion to 133%. Cell content of MCH on days 8, 10, and 12 of culture was 2±1.2, 9±14, 47±12, and 48±6 pg/dish respectively, and this was not significantly changed by any treatment. These results suggest that hypothalamic cells in culture indeed produce MCH, and that this secretion may be regulated by multiple second messenger systems.

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474.15
GLUTAMATE AGONISTS DO NOT INHIBIT MELATONIN PRODUCTION IN THE RAT PINEAL GLAND IN VITRO. L. Ku, R.J. Hando, A. Mendiola. Departments of Neurobiology, Neurology, and Anatomy, Loyola University, Maywood, IL 60153
We have previously described a saturable, stereospecific, pH, time, and temperature-dependent immunoreactive melatonin in vitro that is not inhibited by the glutamate receptor antagonist kainate in the rat pineal gland. The function of this binding site is unknown. In the present study we examined the effect of glutamate agonists on the adrenergic stimulated secretion of melatonin using an in vitro perfusion system (Acusys-APS-10, Endotronics). Pineal glands were perfused with an artificial CSF at a rate of 200 μl/min., and subsequent melatonin release was measured by RIAs. Before drug treatment pineal glands were preincubating for a period of 4 hours by which time melatonin output reached basal levels. The addition of L-glutamate (10-5M) to the perfusion system induced a significant elevation in melatonin release (.005, t-test) at 2200 h and lower levels (p=0.006, t-test) at 2400 h than in saline injected animals. Pineal glands that received these three ISO injections at 1200, 1600 and 1600 h were no different than in saline groups. In conclusion, pineal glands did not release melatonin. It's mechanism of action may be via activating the hypothalamic TIDA neurons. Simultaneous administration of L-glutamate with ISO did not inhibit melatonin production suggesting that glutamate's effects may be mediated via a conversion product (GABA) of glutamate. Supported in part by Sigma Xi, Grants-in-Aid of Research and NSF INBS-86-01726.

474.16
EFFECT OF MULTIPLE INJECTIONS OF ISOPROTERENOL ON PINEAL MELATONIN PRODUCTION IN TURKISH HAMSTERS. B.S. Bengoa, B.O. Rapoport, and A.R. Esterman. The Rockefeller University, New York, NY 10016
In previous studies we found that pineals of the Turkish hamster (Mesocricetus brandti) are responsive to single lactotroph cell level, by means of reverse hemolytic plaque technique. The effect of Glib on free cytosolic calcium ([Ca++]) and adenylate cyclase (AC) activity was also tested. The percentage of PRL-secreting cells was significantly increased by Glib in a dose dependent manner (1μM-10μM). This effect was completely abolished by DA (1μM). The increase of PRL-secreting cells induced by forekolin was not modified by Glib pretreatment, while the dopamine-induced inhibition of PRL was reduced by the blockade of K+(ATP)-channels. Glib neither affected basal AC activity nor modified DA inhibition of the AC enzyme. Finally, Glib induced a dose- and time- dependent membrane depolarization and increased [Ca++] levels but did not prevent DA-induced membrane hyperpolarization and [Ca++] reduction.

474.17
MELATONIN MODULATION OF HYPOTHALAMIC FUNCTION AND ESTROGEN RECEPTOR EXPRESSION (ER) IN LSH/SLAHL HAMSTERS. H. Laxson, B. Wu, C. Galindo, D. Blakey, E. Benjamin, and H.H. Wu. Tulane Univ. Sch. of Medicine, New Orleans, LA 70112, and 1 Univ. of Arizona College of Medicine, Tucson, AZ 85755.
Short photoperiod or exogenous melatonin administration induces a state of anestrus in LSH/SLaHL female hamsters, which is associated with diminished gonadotropin levels. The neuroendocrine control of reproductive physiology is affected by gonadal steroids, particularly estrogen, which controls gonadotropin release through feedback mechanisms mediated by hypothalamic neurons containing ER. We therefore tested the hypothesis that the pineal hormone, melatonin, may alter hypothalamic sensitivity to estrogen by modulating ER expression. Regularly cycling females in long photoperiod (14L:10D) were bilaterally ovariectomized and then given late afternoon injections of melatonin (25 μg/day) or saline for 10 weeks, after which they were sacrificed. Melatonin treatment significantly suppressed FSH, LH, and prolactin serum levels, as determined by RIA. Brain free both groups of animals were analyzed by immunocytochemistry using the human ER monoclonal antibody H222 (Abbott) and DAB. A 50% decrease in the density of ER immunoreactive cells was noted in the medial preoptic area of melatonin treated hamsters, as compared with control animals. These data suggest that melatonin in female hamsters suppresses gonadotropin and prolactin levels, inducing anestrus possibly via its suppression of hypothalamic ER expression.

474.18
EFFECT OF ATP-SENSITIVE K+ CHANNELS ON DOPAMINE INHIBITION OF PRL SECRETION AND SECOND MESSENGER SYSTEMS IN MMQ CELLS: A PITUITARY CLONE NATURALLY EXPRESSING D2 RECEPTORS. O. Neucci, C. Ventura, A. Scorttiello, M. Grimaldi, C. Landolfi. Dept. of Pharmacology, II School of Medicine, University of Naples, ITALY.
In this study we used glibenclamide (Glib) to evaluate the role of ATP-sensitive K+-channels in dopamine (DA) inhibition of PRL secretion measured either in mixed pituitary cultures or at single lactotroph cell level, by means of reverse hemolytic plaque technique. The effects of Glib on free cytosolic calcium ([Ca++] and adenylate cyclase (AC) activity were also tested. The percentage of PRL-secreting cells was significantly increased by Glib in a dose dependent manner (1μM-10μM). This effect was completely abolished by DA (1μM). The increase of PRL-secreting cells induced by forekolin was not modified by Glib pretreatment, while the dopamine-induced inhibition of PRL was reduced by the blockade of K+(ATP)-channels. Glib neither affected basal AC activity nor modified DA inhibition of the AC enzyme. Finally, Glib induced a dose- and time- dependent membrane depolarization and increased [Ca++] levels but did not prevent DA-induced membrane hyperpolarization and [Ca++] reduction.

474.19
Bombesin, a putative peptide neurotransmitter, has been shown to inhibit both basal and stress-induced prolactin. Its mechanism of action may be via activating the hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons. Two doses of 0.15 and 0.75 μg were administered i.c.v. at 1200 h in long-term ovariectomized, estrogen-treated rats. Hourly plasma samples were taken from 1200 h to 1900 h through indwelling intrastratal catheters. The large dose of bombesin completely blocked the estrogen-induced afternoon prolactin surge while the small dose just delayed the surge for an hour. Co-administration with [Leu]-amido[4-Chloro]H-Leu]-bombesin, a bombesin receptor antagonist, no longer effective in blocking the prolactin surge. On the other hand, in bombesin-treated rats, a single injection of sulpiride at 1200 h and at 1400 h also restored the prolactin surge. We conclude from the results that bombesin acts through specific receptor to activate the TIDA neurons and in turn inhibits prolactin secretion. (Supported in part by NSC98-0412-B010-55).

474.20
EFFECTS OF PASSIVE IMMUNONEUTRALIZATION OF PROLACTIN ON THE ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. A.I. Flachenberg, T.W. Toney, C. Gopalan, J.K. Lockwood and K.E. Moore. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824
This study examines the effects of the prolactin (PRL) antisera (raised in rabbits) on tuberoinfundibular dopaminergic (TIDA) neuronal activity as estimated by measuring in the median eminence the concentration of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), or the accumulation of 3,4-dihydroxyphenylalanine (DOPA) 30 min after administration of a decarboxylase inhibitor. Administration of PRL antisera TIDA neuronal activity was decreased in generally intact females but not male rats. PRL antisera had no effect on TIDA neuronal activity in overstimulated female rats, but blocked the stimulatory effect of estradiol (E2) at 2400 h. The effects of E2 on TIDA neuronal activity were modified by DA inhibition of the AC enzyme. Finally, PRL antisera induced a dose- and time-dependent membrane depolarization and increased [Ca++] levels but did not prevent DA-induced membrane hyperpolarization and [Ca++] reduction.
474.21

BOTH FOOTSHOCK STRESS AND DECREASE DECREASE PLASMA PROLACTIN IN MALE HAMSTERS. K.L. HUBMAN, R.H. MOODY, & J.L. MEYERHOFF. PO Box 4010, Dept. of Biology, Georgia State Univ., Atlanta, GA 30302-4010; Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Although stress increases plasma prolactin (PRL) levels in male rats, it was reported that neither handling nor ether exposure affected plasma PRL in male hamsters. We have found increases in the stress-responsive hormones beta-endorphin and cortisol following exposure to acute and repeated social conflict, but only in hamsters that become submissive. We have now examined PRL responses following social conflict (resident-intruder paradigm). For comparison, we have also examined the effect of exposure to footshock stress. Plasma concentration of PRL was measured by RIA using antisera raised against hamster PRL. We found that footshock markedly decreased plasma PRL. For the most part, following both acute and repeated agonistic encounters, plasma PRL was significantly decreased in submissive hamsters. That the PRL response to some stressful stimuli in golden hamsters may be anomalous.

474.22

ESTROGEN AND AN ESTROGEN-BSA CONJUGATE MEDIATE RAPID PROLACTIN RELEASE IN GH3/B6 CELLS. T.C. Pappas, T. J. Collins*, C. S. Watson*, Dept. of Human Pharm. and Genetics, Univ. of Texas, Med. Br., Galveston, TX 77550.

Rapid release of prolactin (PRL) in GH3/B6 cells in response to beta-estradiol (E2) or estradiol conjugated to bovine serum albumin (E2-BSA) was investigated by radioimmunoassay. Attached or serum-supplemented media were treated with steroid or vehicle, and PRL release into the media was assayed. PRL release in cells treated with 10 nM E2 was increased over vehicle controls as rapidly as 1 min. At 5 min, however, there was no significant difference. At 1 min, the PRL release response was saturated at 10 nM E2. E2-BSA was effective in eliciting both 1 and 5 min PRL release over BSA controls at a concentration as low as 1 ng/ml (estimated maximum E2 concentration 0.3 nM), with response saturation at 10 ng/ml; thus, immobilization of the steroid bound at the cell membrane increases the sensitivity of the response. The rapid time course of this PRL release enhancement as well as the ability of the BSA-linked ligand to elicit this response suggests a membrane site of action for estrogen.

474.23

BRAINSTEM ESTROGEN RECEPTOR- AND DOPAMINE-B HYDROXYLASE-IMMUNOREACTIVITY IN MALE AND FEMALE RATS. D.H. Olster, J.D. Blaustein and A.P. Jones. Dept. of Biological Sciences, University of Illinois, Urbana, IL 61801.

We employed the technique of intracranial microdialysis to investigate the mechanism of mCPP-induced prolactin (PRL) release and intravenous blood sampling to investigate the mechanism of mCPP-induced prolactin release. Injections of vehicle, or 0.01, 0.1 or 1.0 mg/kg mCPP, were injected icv into overanesthetized rats 65 h before sacrifice. Subsequently, bromocriptine (BRM0.3 mg/kg, s.c.) or vehicle was injected 50, 30, 20, 14 and 2 h before sacrifice. Four groups were used: 1) vehicle + control, 2) vehicle + BROMO, 3) Rcho cells + vehicle and 4) Rcho cells +BROMO. In vitro TH activity was assessed by incubating hypothalamic explants with brocresine, a dihydroxyphenylalanine (DOPA) decarboxylase inhibitor. The accumulation of DOPA in the medium, which contains nerve terminals for the TIDA neurons, was determined by HPLC. In vivo mCPP treatment was associated with suppression of 60% to 75% of control (7.7±0.6 ng/mg protein/30min) in BROMO-treated rats and was increased 2-fold above control in Rcho- and Rcho+BROMO-treated rats. In a second group, TH mRNA signal levels in the arcuate nuclei, which contain the perikarya of the TIDA neurons, were determined by in situ hybridization with a 35S-labeled cRNA probe for TH. TH mRNA signal levels were markedly reduced in BROMO-treated rats, but this effect was partially reversed in the Rcho+BROMO-treated rats. Serum PRL (ng/ml) was 16.4±2.5 in control rats, and was reduced to 1.0±1.0 or 1.6±0.4 in BROMO- and Rcho-treated rats, respectively. CONCLUSIONS: 1) R cho cells produce a factor(s) which increases TH catalytic activity and is capable of reversing the BROMO-induced suppression of TH activity; in part, by a stimulatory effect on reduced TH mRNA levels; and 2) factor(s) produced by Rcho cells may suppress PRL release, in part, by a dopaminergic mechanism. Supported by HD24190 and HD70306.

474.24

A PLACENTAL FACTOR INCREASES TYROSINE HYDROXYLASE (TH) ACTIVITY IN TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONS. B. K. Bright, M. J. Saper and J. J. Voll. Dept. of Physiology, Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Placental chorion (RCHO) cells, which have morphological features similar to trophoblast giant cells, and produce placental lactogen 1, were used to investigate placental effects on TIDA neuronal activity and prolactin (PRL) release. RCHO cells (100,000 cells/ml) or media, were injected icv into ovariectomized rats 65 h before sacrifice. Subsequently, bromocriptine (BRM0.3 mg/kg, s.c.) or vehicle was injected 50, 30, 20, 14 and 2 h before sacrifice. Four groups were used: 1) vehicle + control, 2) vehicle + BROMO, 3) Rcho cells + vehicle and 4) Rcho cells +BROMO. In vitro TH activity was assessed by incubating hypothalamic explants with brocresine, a dihydroxyphenylalanine (DOPA) decarboxylase inhibitor. The accumulation of DOPA in the medium, which contains nerve terminals for the TIDA neurons, was determined by HPLC. In vivo mCPP treatment was associated with suppression of 60% to 75% of control (7.7±0.6 ng/mg protein/30min) in BROMO-treated rats and was increased 2-fold above control in Rcho- and Rcho+BROMO-treated rats. In a second group, TH mRNA signal levels in the arcuate nuclei, which contain the perikarya of the TIDA neurons, were determined by in situ hybridization with a 35S-labeled cRNA probe for TH. TH mRNA signal levels were markedly reduced in BROMO-treated rats, but this effect was partially reversed in the Rcho+BROMO-treated rats. Serum PRL (ng/ml) was 16.4±2.5 in control rats, and was reduced to 1.0±1.0 or 1.6±0.4 in BROMO- and Rcho-treated rats, respectively. CONCLUSIONS: 1) R cho cells produce a factor(s) which increases TH catalytic activity and is capable of reversing the BROMO-induced suppression of TH activity; in part, by a stimulatory effect on reduced TH mRNA levels; and 2) factor(s) produced by Rcho cells may suppress PRL release, in part, by a dopaminergic mechanism. Supported by HD24190 and HD70306.

474.25

INTRAVENOUS m-CHLOROPHENYLPIPERAZINE INCREASES PLASMA PROLACTIN AND HYPOTHALAMIC SEROTONIN IN CONSCIOUS RATS. J. S. Maguen and L. Butler, Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

While it is generally accepted that m-chlorophenylpiperazine (mCPP) is a dopamine receptor (D1, D2) agonist, this drug also releases 5-HT from brain slices in vitro (Petrie and Williams, 1984). We employed the techniques of intracranial microdialysis and intravenous blood sampling to investigate the mechanism of mCPP-induced prolactin (PRL) release. Injections of vehicle, or 5-HT 100 ng/kg, or mCPP 0.2 mg/kg, were injected icv into anesthetized rats. Dialysate samples were collected at 30 min intervals and immediately assayed for 5-HT by HPLC. Blood samples were withdrawn just prior to and at 30, 60 and 90 min after drug (or vehicle) injection. Plasma was assayed for PRL by RIA. Plasma PRL was increased over baseline by mCPP 5-HT, and PRL release was not altered by iv saline (1.0 ml/kg). Pretreatment with the 5-HT antagonist 5,7-DHT (15 ng/kg) blocked the mCPP-induced rise in dialysate 5-HT, but did not reverse the stimulatory effect of mCPP on plasma PRL. Phentolamine alone elicited a modest 2-fold increase in dialysate 5-HT without affecting plasma PRL. Our results suggest that mCPP enhances extracellular 5-HT release by a presynaptic mechanism involving the 5-HT uptake carrier. The PRL-releasing action of mCPP appears to be independent of the presynaptic drug effects. (Supported by the Bureau of Biological Research, Rutgers Univ.)

474.26

PROLACTIN RELEASE IN GOLDEN HAMSTERS IS UNDER DOPAMINERGIC INHIBITION. T.O. Moore, K.L. Hubman, R.H. Moody and J.J. Swanson, Neuroendocrinology Branch, Dept. of Medical Neuroscience, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100. P.O. Box 4010. Georgia State University, Atlanta, GA 30307-5100.

Exposure to stressors produces either no effect or decreases plasma levels of prolactin (PRL) in male hamsters. This is in contrast to the response in the rat, in which stress increases plasma PRL. As a first step in exploring the neuroendocrine regulation of PRL secretion in hamsters in vivo, we sought to establish whether PRL secretion was under dopaminergic inhibition. Accordingly, we treated hamsters with domperidone, a peripherally-active dopamine receptor blocker which reaches the anterior pituitary gland and has been used successfully to block dopaminergic inhibition of PRL release. Injections of vehicle, or 0.01, 0.1 or 1.0 mg/kg of domperidone were given intraperitoneally to male golden hamsters. One hour after the injections, trunk blood was collected in heparin, centrifuged and plasma frozen. Plasma PRL was measured by RIA, using antisera raised against hamster PRL. Domperidone elicited dose-related increases in plasma PRL, reaching 130 ng/ml at the highest dose. Thus, although the PRL response to stress in male hamsters is anomalous compared to the rat, its release does increase rapidly after blockade of dopaminergic inhibition.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
474.28 RELAXIN IN THE CEREBROSPINAL FLUID OF FEMALE RATS. L.J. Parry, B.S. Poterski*, C. Wansidge*, A.J.S. Summerle. Biomedical Sciences, Ontario Veterinary College, Guelph, Ontario, N1G 2W1, Canada.

Experiments were done to investigate whether the 6kD peptide hormone relaxin (RXN) injected into the peripheral circulation, enter the CSF in uroeh-anesthetized mice. Mice were injected with either 5ng (RXN) (in 0.1ml saline: n=64) or saline (0.1ml: n=61) into the caudal artery. A single 100μg sample of CSF per rat was withdrawn from the cisterna magna at either 0, 5, 10, or 20 mins following treatment; samples were pooled from each group. Porcine RXN concentrations were measured in the pooled CSF samples (n=6/group) by specific RIA (O’Byrne & Steinetz, 1976) RXN1 was not detected in the CSF either before treatment or in CSF samples from saline-treated animals. In contrast, significant (P<0.05:ANOVA) elevations in CSF RXN concentrations were observed in all RXN-treated animals. The presence of RXN in CSF samples was confirmed by SDS-PAGE separation of the CSF samples. Porcine RXN was identified only in CSF samples of RXN-treated animals by Western Blot analysis using a porcine RXN antibody (supplied by DG Porter). These data are the first demonstration that peripherally circulating RXN enters the CSF despite its 6kD molecular weight. Supported by NSERC (Canada) and OMAF.


It is well known that acetylcholine stimulates insulin secretion. We have used a reverse hemolytic plaque assay to study the effect of carbachol (CCh) in cultured rat B-cell, in different glucose concentrations. CCh (10 nM) increased the percentage of plaque-forming cells in 5 and 10 mM glucose by 85 and 81%, respectively. However, in 15 mM glucose it decreased in 18% (n=4).

Recently, we described functional subpopulations of B-cells, based on the amount of insulin secreted in single cells (Hiriart and Ramírez-Medel, Endocrinology, in press). We classified them as: small (SP), medium (MP) and large (LP) plaque-forming cells. In 5 mM glucose, mainly SP and MP cells were detected. CCh increased the percentage of MP cells and recruited LP cells. In 10 mM glucose, the three subpopulations were observed, which increased the percentage of MP and LP cells. In contrast, CCh in 15 mM glucose decreased the percentage of MP and LP cells.

The results suggest that the effect of CCh in low glucose is greater, because more cells are recruited to secreting activity. Moreover, B-cells subpopulations with higher secretion rates may be preferentially stimulated by carbachol.

474.30 PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS AND REGULATION OF STEROIDGENESIS IN RAT BRAIN MITOCHONDRIA. L.D. McCaulley*, N.C. Lan, M.M. Tonmey*, J.C. Hawley, K.W. Gee*, Department of Molecular Pharmacology and Toxicology, Children’s Hospital, University of Southern California, Los Angeles, CA 90033. Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Recent evidence has suggested that steroid biosynthesis may occur in central nervous system tissue. Increased pregnenolone synthesis can be induced with a benzodiazepine (BZ) receptor located on the outer mitochondrial membrane. To evaluate the synthetic capabilities of brain mitochondria, we fractionated Sprague-Dawley rat brains, ligands with varying affinities for the peripheral-type BZ receptor on the mitochondrial membrane were examined for their ability to stimulate pregnenolone production from cholesterol. Dexamethasone, 30 ng 4646 and PK 11195 increased steroidogenesis by at least two-fold whereas clonazepam had no significant effect. In addition, the ability of steroidalgenesis activator peptide (SAP) and three partial fragments of dexamethasin binding inhibitor were evaluated for activity. All peptides significantly stimulated cholesterol conversion to pregnenolone in brain mitochondrial preparations. Furthermore, SAP displayed competitive kinetics against Ro 4646 as determined by Scatchard analysis. The Kd for [3H]Ro 4646 binding in the presence of SAP was calculated to be 5.69 nM, about twice that of control [3H]Ro 4646 binding. These results suggest that the brain may be capable of de novo synthesis of steroids and that peptides and small molecules such as Ro 4646 may share a common site of action. (Supported by NIH grants NS25896 and NS24645).

475.1 INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN (IRAP) EQUALLY INHIBITS IL-1α AND IL-1β-INDUCED ACTH SECRETION AND THYMCYTIE PROLIFERATION. G. Marini, K.M. Linsert* and B.M. Harper*, Endocrine-Neuromus Res Lab, Dept of Medicine, Hennepin Cnty Med Ctr and U of MN, Minneapolis, MN, USA.

Injection of IL-1 (IL) stimulates the release of ACTH via a central mechanism. Whether apparent differences in efficacy of IL-1α vs IL-1β due to different receptors has not been clarified. We sought to address this from a functional perspective, by examining the inhibition by IRAP of ACTH secretion and transcription from the ACTH gene. We examined the single step synthesis of histamine from L-histidine. To study the effects of IRAP on the neuroendocrine system we determined the IC50 of the cat histidine decarboxylase gene with HCl (PMAS 87:7133, 1990) as probe and have identified the transcriptional start site by primer extension. Sequence data have revealed a putative promoter region which includes a TATA-like element, and sequences resembling hormone responsive elements. Experiments were conducted to determine whether the IL-1 gene is regulated by estrogen.

Sprague-Dawley rats were ovariectomized and after 14 days were treated with 10μg of estradiol in 100μl sesame oil. Whole brains were removed and processed for in situ hybridization, Northern blot analysis and enzyme assay. After estradiol treatment, both the mRNA and HDC activity were elevated as compared to vehicle only controls. Experiments are in progress to define the level at which regulation occurs. (Supported by grants from the North Carolina Biotechnology Center and Glaxo Pharmaceuticals.)


Some of the activities of IL-1 are known to be mediated by IL-6. Intracerebroventricular injection of IL-1 enhances the production of 40 ng of human recombinant 8 IL-1 results in a more marked induction of IL-6 compared to systemic injection. This effect is antagonized by the i.e.v. administration of the IL-1 receptor antagonist (IL-1ra, 20 μg). Injection of IL-6 by IL-1a is greatly attenuated by naloxone (10 mg i.p.) pretreatment and by 6-OHDA treatment. IL-6 synthesis can be induced via a benzodiazepine (BZ) receptor located on the outer mitochondrial membrane. To evaluate the synthetic capabilities of brain mitochondria, we fractionated Sprague-Dawley rat brains, ligands with varying affinities for the peripheral-type BZ receptor on the mitochondrial membrane were examined for their ability to stimulate pregnenolone production from cholesterol. Dexamethasone, Ro 4646 and PK 11195 increased steroidogenesis by at least two-fold whereas clonazepam had no significant effect. In addition, the ability of steroidalgenesis activator peptide (SAP) and three partial fragments of dexamethasin binding inhibitor were evaluated for activity. All peptides significantly stimulated cholesterol conversion to pregnenolone in brain mitochondrial preparations. Furthermore, SAP displayed competitive kinetics against Ro 4646 as determined by Scatchard analysis. The Kd for [3H]Ro 4646 binding in the presence of SAP was calculated to be 5.69 nM, about twice that of control [3H]Ro 4646 binding. These results suggest that the brain may be capable of de novo synthesis of steroids and that peptides and small molecules such as Ro 4646 may share a common site of action. (Supported by NIH grants NS25896 and NS24645).

475.3 NEURAL-IMMUNE INTERACTIONS: INTERLEUKINS AND NEURAL FUNCTIONS.

Receptors for IL-1β are expressed in rat hypothalamic neurons. Earlier studies have shown that IL-1β released by immune cells can alter the physiological function of this CNS neuronal type. (Supported by DK 26741 and the MRC of Canada)

475.4 INTERLEUKIN-1-BETA INCREASES INTRACELLULAR CALCIUM AND ALTERS THE ELECTROPHYSIOLOGY OF CULTURED CEREBELLAR PURKINJE CELLS IN VITRO. N. Jr., J. L. Tauli*, A. S. Urata* and D. L. Gruid. Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Interleukin-1-beta (IL-1β) produces a depression of membrane excitability in vitro. The calcium influx caused by IL-1β is blocked by neuropeptides and antagonists. (Supported by NIH HD 11362)


The cytokine interleukin-1 beta (IL-1β) has been proposed as a messenger between the immune system and the central nervous system (CNS). IL-1β is released by monocytes, macrophages and activated astrocytes and ameboid microglia during CNS injury. IL-1β binding sites are distributed heterogeneously in the CNS and are particularly densely concentrated in the cerebellar cortex (Farrar et al., 1987, 139:459). This study examined the effects of local application of IL-1β on the firing rate of cerebellar Purkinje cells (PC) in chloral hydrate- or urethane-anesthetized rats. IL-1β (1.5 nM, 0.4 - 4.8 pg) applied to the brain surface produced a depression of PC firing that recovered over time. This inhibition was observed in 77% of the PC that were tested and resembled the inhibition that was produced by microinjection of hypothalamic CRF. Equivalent ejections of the vehicle control solution had no effect on PC firing. These results indicate that IL-1β can alter neuronal excitability in a region of the CNS that contains IL-1β binding sites, and support the hypothesis that IL-1β may have a neuromodulatory effect in the CNS.


We have reported that central administration of the cytokine, IL-1β, blocked the steroid-induced LH surge in ovariectomized rats apparently due to decreased hypothalamic LHRH secretion via an increase in endogenous opioid activity. To determine the component of LH episodes responsible for the decrease in LH levels, we examined the effects of peripheral IL-1β administration on LH release. Immunoreactive LH secretion was significantly decreased in the arcuate nucleus (ARC) of the hypothalamus. In contrast, injection of IL-1β did not measurably alter c-fos expression in the PVN or in the arcuate nucleus. These results suggest that c-fos induction in the external zone of the median eminence is a major mechanism to stimulate the HPA axis activity, while icv administered IL-1β activates neurons within the PVN.

475.7 INVOLVEMENT OF THE EPOXIDE PATHWAY IN INTERLEUKIN-6 (IL-6) STIMULATED CORTICOTROPIN-RELEASING FACTOR (CRF) SECRETION IN VITRO. J. L. Tauli*, J. L. Tauli*, R. M. M. McCann. Department of Physiology, Neuropeptide Division, U.T. Southwestern Medical Center, Dallas, TX 75235-9080.

Since we demonstrated that the epoxigenase (EPO) pathway of arachidonic acid metabolism system may mediate IL-6-stimulated CRF release in vitro, we decided to examine the influence of clotrimazole (CLO), a specific EPO inhibitor, on IL-6-stimulated CRF release from the rat medial basal hypothalamus (MBH) pretreated with CLO followed by IL-6 administration to the MBH. CLO (50 μg/g MBH); 100 μg IL-6 (50 μg/g MBH); 250 μg IL-6 (50 μg/g MBH); 500 μg IL-6 (50 μg/g MBH) were injected to the MBH pretreated with CLO (50 μg/g MBH) or CLO (50 μg/g MBH) and CLO (50 μg/g MBH). CRF release was measured using RIA. None of the CLO concentrations used changed basal CRF release significantly. As reported previously, 10 μg IL-6 evoked increased CRF release which was significantly suppressed by CLO in a dose-dependent manner at concentrations ranging from 10 μg IL-6/g MBH to 5 μg IL-6/g MBH. The maximal inhibitory effect was observed with 100 μg CLO/kg MBH, 50 μg IL-6/g MBH, 250 μg IL-6/g MBH and 500 μg IL-6/g MBH. The results suggest that IL-6-stimulated CRF release from the MBH in vitro by the activation of the EPO pathway.


We have reported that central administration of the cytokine, IL-1β, blocked the steroid-induced LH surge in ovariectomized rats apparently due to decreased hypothalamic LHRH secretion via an increase in endogenous opioid activity. To determine the component of LH episodes responsible for the decrease in LH release, we examined the effects of IL-1β on pulsatile LH secretion in castrated male rats. IL-1β was injected either icv or iv in 0.4 pg or 4.8 pg doses. Plasma LH levels were determined by RIA. No significant differences were observed in LH levels between the control and the IL-1β treated group. These results suggest that IL-1β administration does not affect LH release.
475.9
INTERLEUKIN-1 (IL-1)-INDUCED INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) EXPRESSION IS BLOCKED BY RECEPTOR ANTAGONIST (IL-1ra). L. Hong*, M. R. Opp, and J. M. Krueger. University of Tennessee, Memphis, TN 38163

ICAM-1 is a cellular ligand that binds lymphocyte function-associated antigen-1 and mediates lymphocyte-tissue cell contact (1). ICAM-1 expression is important in inflammatory responses and is regulated by cytokines such as IL-1. Interleukin (IL-1) is a protein in the tumor necrosis factor-alpha (TNF-α) superfamily (1). To study ICAM-1 expression in brain cells, we examined ICAM-1 expression in a human glioblastoma cell line (HT1B) and a normal brain homogenate by ELISA to quantify ICAM-1. Cells were incubated for 4 to 6 days with various concentrations of recombinant human IL-1β (rIL-1β), rhIFN, and rhTNF in the absence or presence of IL-1ra (1 or 10 μg/ml). rhIL-1β, rhIFN, and rhTNF potentiated ICAM-1 expression in both cell lines in a dose-related manner. The effect of rIL-1β on ICAM-1 expression was more potent in HT1B cells, while that of rhIFN was more potent in HT11 cells. rhIL-1β-induced ICAM-1 expression, but not rhTNF, was completely inhibited by 10 μg/ml of IL-1ra. Our findings also indicate that expression of ICAM-1 by neuronal cells is regulated by cytokines, thus suggesting neuronal cells are also a target of binding to lymphocytes and could be involved in inflammatory responses.


475.11

To estimate whether changes of neuronal activity could alter the synthesis of interleukin-1β (IL-1β) in the brain, the influences of convulsion evoked by drugs on the mRNA level of IL-1β were studied in the rat. Male Sprague-Dawley rats were injected with kainic acid (KA; 10 mg/kg i.p.) or pentyleneetetrazol (50 mg/kg i.p.). The mRNA level of IL-1β mRNA in various brain regions, that is, the cerebral cortex, hippocampus, striatum (STR), thalamus (THL), hypothalamus (HT), midbrain (MB), pons-medulla (PM) and cerebellum (CEB), was examined by northern blot analysis. IL-1β mRNA was induced by KA intensely in the CC, THL and HT, moderately in the HPP and weakly in the MB and CEB. The increase in IL-1β mRNA was not observed by the injection of KA. In situ hybridization study confirmed the regional difference in the induction of IL-1β mRNA by KA.

These results provide the evidence that intense neuronal excitation induces IL-1β mRNA in particular regions of the brain.

475.12

The present study was undertaken to determine the effects of interleukin-1β (IL-1β) on renal sympathetic nerve activity (RSA), arterial blood pressure (AP) and heart rate (HR) as well as body temperature in conscious rats. Either intravenous (i.v.) or intracerebroventricular (i.c.v.) administration of IL-1β elicited increases in AP, HR and RSA accompanied by a rise in body temperature. The maximum changes in AP, HR and RSA occurred 10-15min after i.v. injection of IL-1β (10μg). 20-25 min after i.c.v. injection (5ng). The responses induced by the i.v. and i.c.v. injections lasted for about 15-30 min and were completely abolished when the animals were pretreated with a cyclo-oxygenase inhibitor, indomethacin (i.v., 10mg/kg). Moreover, i.c.v. injection of prostaglandin E2 (1μg) produced responses similar to those induced by IL-1β but with a rather short latency. The results suggest that IL-1β augments cardiovascular and sympathetic outflow through the central action of prostaglandin E2 in conscious rats. (This work was entrusted to University of Occupational and Environmental Health, using the Special Coordination Funds for Promoting Science and Technology.)

475.13
INTERLEUKIN-1 (IL-1) RECEPTORS IN THE BRAIN-ENDOCRINE AXIS CORRESPOND TO TYPE I BUT NOT TYPE II RECEPTORS: EVIDENCE FROM AFFINITY CROSS-LINKING STUDIES. D.E. Grigorian, T. Takao, R.C. Newton* and E.B. De Souza. The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880 and NIDA Addiction Research Center, Baltimore, MD 21224.

IL-1 is a key mediator of the immune response to stress, infection and antigenic challenge. IL-1 also alters neuroendocrine activity through induction of both brain and direct actions in endocrine tissues. IL-1 receptors have been identified in brain and in endocrine organs with similar pharmacological characteristics to IL-1 receptors in the T-lymphoid cell line EL-4.6. Recently, multiple IL-1 receptors, referred to as type I (M.W. = 60 kDa) and type II (65 kDa), have been isolated in T- and B-lymphoid cell lines, respectively. In the present study, we have used chemical affinity cross-linking of 125I-recombinant human IL-1α (125I-IL-1α) followed by SDS-PAGE and autoradiography to compare the apparent M.W. of IL-1 receptors in the brain-endocrine-immune axis to that in T- and B-lymphoid cell lines and to mouse and rat brains. 125I-IL-1α was incubated in mouse serum homogenates into two specific and distinct proteins corresponding to the respective M.W. of type I and type II receptors. The on the other hand, 125I-IL-1α was incubated in rat brain homogenates in T-cells and B-cells and in T- and B-lymphoid cell lines, respectively. These data demonstrate a similarity between IL-1 receptors in the brain-endocrine axis and type I IL-1 receptors in T-lymphocytes and suggest the importance of the cytokine in coordinating brain-endocrine-immune function.

475.14
TYPE I INTERLEUKIN-1 (IL-1) RECEPTORS IN THE MOUSE BRAIN-ENDOCRINE-IMMUNE AXIS LABELED WITH 125I-RECOMBINANT HUMAN IL-1 RECEPTOR ANTAGONIST. T. Takao, R.C. Newton* and E.B. De Souza. The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880 and NIDA Addiction Research Center, Baltimore, MD 21224.

The cytokine IL-1 has a variety of effects in brain including induction of fever, alteration of slow-wave sleep and alteration of neuroendocrine activity through direct actions in brain and through actions in endocrine tissues. IL-1 receptors have been identified in brain and in endocrine organs with similar pharmacological characteristics to IL-1 receptors in the T-lymphoid cell line EL-4.6. Recently, multiple IL-1 receptors, referred to as type I (M.W. = 60 kDa) and type II (65 kDa), have been isolated in T- and B-lymphoid cell lines, respectively. In the present study, we have used chemical affinity cross-linking of 125I-recombinant human IL-1α (125I-IL-1α) followed by SDS-PAGE and autoradiography to compare the apparent M.W. of IL-1 receptors in the brain-endocrine-immune axis to that in T- and B-lymphoid cell lines and to mouse and rat brains. 125I-IL-1α was incubated in mouse serum homogenates into two specific and distinct proteins corresponding to the respective M.W. of type I and type II receptors. The on the other hand, 125I-IL-1α was incubated in rat brain homogenates in T-cells and B-cells and in T- and B-lymphoid cell lines, respectively. These data demonstrate a similarity between IL-1 receptors in the brain-endocrine axis and type I IL-1 receptors in T-lymphocytes and suggest the importance of the cytokine in coordinating brain-endocrine-immune function.

475.10
CENTRAL IL-1B INDUCES A RISE IN BLOOD PRESSURE IN ANAESTHETIZED MICE. RS Poterski*, AJS Summerlee. Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario Canada N1G 2W1.

We previously reported that central IL-1B postimplantation pregnancy loss in mice (Croy et al., PNEI 1990; 3: 242-250). Preliminary data indicated that i.c.v. IL-1B causes a rise in systemic blood pressure leading to disruption of blood supply to the developing fetus. We tested whether injection of IL-1B (Genzyme, Boston, MA) into the cerebral ventricles of CD-1 non-pregnant mice (7-8 weeks old) would cause a pressor effect. Mice were anaesthetized with tribromoethanol i.p. and the left common carotid artery cannulated for direct recording of blood pressure. Each animal was then placed into a stereotaxic apparatus and the hole drilled above the left lateral cerebral ventricle. A 10 μl microsyringe was placed with its tip in the middle. The effects of IL-1B (1 ng i 1 μl) i.e.v. were compared with control injections of 1 μl PBS-BSA (vehicle) i.e.v. One minute after injection of IL-1B, there was a significant (P<0.05) rise in systolic and diastolic blood pressure (mode 16-20 mm Hg; range 5-26 mm Hg) in all animals (N/9). In contrast, no change in blood pressure was seen after PBS-BSA i.e.v. The data indicate that rIL-1β i.e.v. causes a consistent pressor effect in mice.

Supported by NSERC (Canada) & OMAF.

Interleukin-1 (IL-1) receptor mRNA, which has been shown to affect hypothalamic and pituitary hormones, notably with resulting increase in corticosterone production. These studies were carried out in order to determine the occurrence of IL-1 receptor (IL-1R) in the rat pituitary. Antisera raised in rabbits against synthetic peptides of the murine lymphocyte IL-1R were used in immunohistochemical staining of the cells of the intermediate lobe of both mouse and rat pituitary. In addition, a population of the cells in the anterior lobe were mIl-1R immunoreactive. PCR analysis using primers for human and mouse lymphocyte IL-1R confirmed the synthesis of this protein in the pituitary. Experiments using in situ hybridization histochemistry to determine the cellular localization of IL-1R mRNA in the pituitary and to investigate its regulation are in progress.

These findings add morphological and biochemical support to the functional evidence of IL-1 receptors in the pituitary, although the source of IL-1 acting on these receptors remains unclear. This work was supported by grants from the Swedish MRC.


Immunocytes are now known to regulate neurotransmitter gene expression. Our laboratory has recently shown that in explant cultures of superior cervical (sympathetic) ganglia (SCG), the macrophage product interleukin-1 (IL-1) dramatically increases both the levels of substance P (SP) and the release of its prohormone precursor, proenkephalin (PPT). We have now examined the products of activated macrophages, including tumor necrosis factor-alpha (TNF-alpha) and IL-1. While human macrophages released SP into 20 U/ml produced a modest doubling of SP, hrIL-1 had no effect on SP release by these authors. Lipo polysaccharide (LPS, Lgpg/ml) from E. coli, a strong activator of the immune system, caused a two- to five-fold increase in SP that was inhibited by an IL-1 receptor antagonist (Synergen). These data suggest that SP induction by LPS may be mediated through the production of IL-1 in the culture. In the testing of the T-cell products on SP expression, we found that doses as high as 20 U/ml of hrIL-1 (Collaborative Research) or 800 U/ml of murine recombinant IL-4 (Genzyme) failed to alter SP levels. However, in the presence of LPS, a common co-stimulant with other immune cytokines, IL-4 increased SP levels above those induced by LPS alone. These data taken together provide further evidence for a regulatory interaction between the immune and nervous systems. (Supported by a grant from the ONR.)


Lymphokines, in particular the interleukins (ILs), can be released from microglia and astrocytes in culture. However, the effects of ILs on the function of neuronal and glial cells in vivo is not completely understood. The present study characterized the effects of various lymphokines on microglial and astrocyte function in culture, by measuring the uptake of ['H]thyminine and the release of IL-1 and IL-2 from these cells. Furthermore, the effects of these lymphokines on the survival of hippocampal neurons in vitro was also assessed. Our results show that IL-1, -2, and other lymphokines enhanced the uptake of ['H]thyminine (by 47-88%), as well as the release of IL-1 and -2 (by 25-125%) in microglial cells in culture. In addition, several 'classical' growth factors (GFs) such as NGF, PCF and EGF also increased both measures. Similar results were obtained for astroglial cultures. In hippocampal neurons, TNF-beta, IL-1 and IL-2 significantly increased GF synthesis; both neurotrophic and neurotoxic effects were observed. These results suggest a complex relationship between lymphokines and GF function. This in turn may further exacerbate the effects of lymphokines. (Supported by grants from the ONR.)
475.21 IMMUNOCHEMICAL LOCALIZATION OF INTERLEUKIN-4 (IL-4) IN BRAINS OF BALB/c, NUDE AND SCID MICE. I. Steri, N. Sadayavan, D. K. Kini and D. K. Kini. Dept. of Physiology, Univ. of Texas, Southwest, Med. Center, Dallas, TX 75235-9060.

Monoclonal antibody 11B11 rat anti-mouse rIL-4 (Obara & Paul, Nature, 325:537, 1987) was used with biotinylated protein-G (BPG-Zymed Labs) and the ABC Elite kit (Vector Labs). In order to enhance the final reaction product, the sections were additionally rechromogened with diaminobenzidine and counterstained. In lymph nodes from control and anti-IgG (induces IL-4) injected mice also disappeared. Although individual patterns varied, with exceptions of the fornix were immunostained. Intense punctate staining was also associated with neuronal perikarya and proximal segments of their fibers in field CA3 of the hippocampus, supracallosal and endopiriform layers of the cortex, olfactory stria and nuc triangularis septi (NTS). Non-neuronal staining occurred in astrocytes, microglia and blood vessels. In the amygdaloid nucleus, the frequency and intensity of staining in most brain regions were greatly reduced. In contrast to BALB/c mice, CA1 stained more than CA3 and more microglia were negative in the NTS. In the severe combined immunodeficient (SCID) mouse there was no staining of brain structures. Therefore, the degree of immunostaining in both of these models determines the presence of IL-4 in brain. Supported by AA-06014.


The human immunodeficiency virus (HIV) binds to the cell surface molecule CD4 and, once internalized, induces expression of a cell-surface glycoprotein (gp-120) that is essential for virus replication. Under controlled conditions, infection can be used to determine the relative presence of CD4 in brain. A thick section was pretreated with 0.1% triton. Monoclonal antibody 12G5 rat anti-mouse (BFG and ABC Prior to color development. Staining for IL-4 disappeared when sections were incubated in a mixture of purified 11B11 added to rIL-4 serum. Similarly, staining in lymph nodes from control and anti-IgG (induces IL-4) injected animals was also observed. Although individual patterns varied, with exceptions of the fornix were immunostained. Intense punctate staining was also associated with neuronal perikarya and proximal segments of their fibers in field CA3 of the hippocampus, supracallosal and endopiriform layers of the cortex, olfactory stria and nuc triangularis septi (NTS). Non-neuronal staining occurred in astrocytes, microglia and blood vessels. In the amygdaloid nucleus, the frequency and intensity of staining in most brain regions were greatly reduced. In contrast to BALB/c mice, CA1 stained more than CA3 and more microglia were negative in the NTS. In the severe combined immunodeficient (SCID) mouse there was no staining of brain structures. Thus, the degree of immunostaining in these mutant animals parallels the relative presence of brain CD4. Supported by AA-06014.

NEURAL-IMMUNE INTERACTIONS: STRESS AND BEHAVIOR

476.1 CAMP AND INTRACELLULAR CALCIUM LEVELS IN SPLENIC MONOCYTOELLS FROM STRESSED RATS. R.A. Bonnenfeld and E.L. Rabin. Brain Behavior and Immunology, University of Pennsylvania, Philadelphia, PA 19104.

Previous findings have implicated norepinephrine as the neurotransmitter involved in stress-induced splenic lymphocyte immunosuppression. Although work by others indicates that a variety of stimulants of CAMP tend to suppress DNA synthesis, our findings indicate that this mechanism is not a likely explanation for the stress-induced immunosuppresion induced by stress. The CAMP levels from cells taken from stressed rats significantly were not elevated in resting cells. Additional, when cells were stimulated with Con-A for various amounts, essentially no differences were seen in a CAMP levels between stressed and control lymphocytes. However, initial calcium levels in cells from stressed animals were found to be significantly higher than levels in cells from control animals. In addition, the response by fura-2 loaded cells to stimulation with Con-A tended to be different for cells from shocked versus cells from control animals. Further studies are in progress to define the mechanism of stress-induced mitogenic suppression.

476.2 IL-1 EFFECTS ON STRESS-RESPONSIVE AND NON-RESPONSIVE CRH NEUROSECRETORY AXONS IN RATS. M.L. Whitall, R.S. Perlstein*, E.H. Mougèy1 and B. Petri. Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5145, and 1Walter Reed Army Institute of Research, Washington, D.C. 20075-5100, USA.

VP-containing, stress-responsive and VP-deficient, stress-nonresponsive CRH axon subtypes were identified by EM immunocytochemistry, which was also used to monitor axon activation (vesicle depletion) after a 10 μg IL-1β, 10 μg IL-1α, 10 μg IL-1α, or 10 μg IL-1β, into 450 Sprague Dawley rats, there were similar increases in plasma ACTH (3 times control) and decrease in prolactin (to 30% of control). Endotoxin (LPS, 0.1 or 1 mg) produced comparable effects. One μg IL-1α did not affect ACTH levels. Five but 10 μg IL-1β, both VP-containing and stress-responsive axons, were seen in immunocytochemistry and not in control. Staining for IL-1α was seen in subtypes (4% for both subtypes). Selective depletion was also seen after 1 mg LPS. Vesicle depletion after 10 μg IL-1α was less consistent, perhaps due to a lower affinity of IL-1α to IL-1 receptors mediating the response. The results demonstrate that the first time that, unlike stress, IL-1 can activate both the VP-containing and the VP-deficient subtypes of CRH axons. In contrast to recent suggestions in the literature, we conclude that the ACTH response to IL-1 involves stimulation of pituitary corticotropes by both VP and VP.

476.3 EFFECTS OF SIGNALLED AND UNSIGNALLLED STRESS ON NATURAL KILLER CELL ACTIVITY. J. Erwin and C. Netter*. Psychology Dept., Queen’s University, Kingston, Ont., K7L 2Y2.

A number of studies have shown that the stress-induced immunosuppression may be linked to the modulability or predictability of the stressor. In the present study we assessed whether the presentation of a warning signal prior to stress exposure would attenuate or enhance the stress-induced immunosuppression. NK activity was assessed in male C57 mice following exposure to one of two stress conditions. For mice in the Signalled Stress group, 6 sec footshock trials (150μAmp, 60 sec interval) were preceded by a 10 sec light cue (1000μW, 3.574 V). Twenty-four hours later, 6 sec footshocks were administered. The light-tone CS presentations were presented on a random schedule. The Not-signalled Stress group included control for any effects from the light-tone CS itself. To establish the association between the signal and the aversive stimulus, the Signalles Stress group was exposed with 10 CS footshocks, followed by a day of 60 CS footshocks. The Not-signalled Stress group also received 10 footshock trials over 5 days. On the sixth day both groups received 60 trials of stress exposure and controls received an equivalent amount of stress. Twenty-four hours later, the splenic NK activity was assessed with a chromium release assay. Mice exposed to the CS alone or to Unsignalled shock exhibited a modest reduction in NK activity, suggesting that both conditions were mildly stressful. However, mice in the Signalled Stress condition showed a marked suppression of NK activity. Contrary to the view that a warning signal allows animals to adopt preparatory responses to buffer the impact of the stressor, these data indicate that the signal increases the stressiness of the situation.

476.4 STRESSOR-INDUCED C-FOS EXPRESSION IN BRAIN STEM LOCI OF THE RAT: A CORRELATION OF NEURAL ACTIVATION WITH IMMUNE ALTERATION. M.A. Perenson, D.B. Pearson, D.S. Perenson, K.M. Perenson, and D.S. Perenson. Dept. of Pathology and Biology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Physiological aversive stimuli, such as mild stress, can suppress the mitotic responsiveness of peripheral blood and splenic lymphocytes and depress splenic natural killer cell activity. In addition, these same immune alterations are associated with aversive, conditioned stimuli when paired with aversive stimuli. In order to define areas of the brain that influence immune function and respond to stressors, we have previously identified stress-induced-C-Fos activated areas of the diencephalon. In accordance with this model, we found that the C-Fos was cytoplasmically expressed in cells of the paraventricular nucleus (somatostin contain CRH and CRH) and other hypothalamic areas directly associated with autonomic function. In this study, C-Fos induction in the brain stem of the rat was examined in control, shocked, and conditioned animals. C-Fos was strongly expressed in the lateral habenula (AH), the nucellar of the solitary tract (A2/C2), the ventral lateral medulla (A1/C1), and the paraaqueductal gray in response to the conditioned stimulus (US, electric footshock). Conditioned animals showed C-Fos induction in these same areas but to a lesser degree. Control animals exposed to a conditioned stimulus (CS, electronic tone) in the absence of the US, expressed very little, if any, C-Fos activity in the above loci, except for a small degree of expression in the periaqueductal gray matter. These results further confirm the role of autonomic and endocrine pathways as mediators of the stress response and will help to more fully characterize the pathways of stress-induced immune alteration.

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476.5 **STRESS-INDUCED ALTERATIONS IN INTERFERON PRODUCTION AND IA ANTIGEN EXPRESSION: ATTEMPTION BY A D-ADRENERGIC RECEPTOR ANTAGONIST**


Previous work in this lab has demonstrated that the physical stress of electric foot-shock can alter splenic mitogen response to Con A and splenic NK activity. However, this stressor does not alter TH1 or TH2 cytokine response. The present study examined the effects of foot shock on the production of another T-cell produced lymphokine, IFN-gamma. IFN-gamma was induced by Con A stimulation of splenocytes for 48 hr. The splenocytes from shocked rats produced 50% less IFN-gamma as measured by ELISA. The reduction in IFN-gamma could be attenuated in a dose dependent manner by administration of naloxol (a gift from C. Rabin) prior to shock. Cell surface expression of class II antigen was also found to be reduced in splenocytes from shocked rats. Expression of class II antigen on macrophages is mediated by IFN-gamma and is important in cell-cell interactions.

476.7 **IMMUNOSUPPRESSION IN MICE INDUCED BY ISOLATED HOUSING: ANTAGONISM BY DIAZEPAM**


Noninbred CD-1 mice transferred from isolated housing to group housing exhibited a decrease in the peak plaque forming cell (PFC) response and antibody titer observed 4 days after inoculation with sheep red blood cells (SRBC: 10^6). This effect was not due to the transfer of housing condition, but instead was a consequence of the isolation. Moreover, the measured immune response was dependent on the duration that mice were isolated prior to inoculation. The maximal decline of the immune response appeared in mice that received 5 to 10 days of isolation, after which the PFC response and antibody titers began to increase, approaching the values of group housed mice. Treatment with diazepam (1.0 mg/kg) for 5 days prior to inoculation and throughout the 4 day period following antigen treatment, antagonized the effects of the isolation treatment. These data suggest that the immunosuppression may have resulted from the anxiety or arousal stemming from the isolation.

476.8 **DEPRESSION, STRESS, AND IMMUNE FUNCTIONING IN PREPUBESCENCE CHILDREN: EFFECTS ON PHAGOCYTOSIS**


Previous work has shown there to be an age related effect of depression on the immune system, but no studies have assessed this association in prepubescent children. This study was designed to study the effects of stress and depression on immune functioning in this age group. A total of 48 children, age 9-12 years, were divided into three groups: non-depressed children with intact (I) and non-intact (NI) families, and children with major depressive disorder (MD). The depressed subjects were matched to controls in terms of age, sex, SES, and race. Depressed children showed a decrease in phagocytic function as measured by ingestion and killing of opsonized zymosan particles. Patients showed a decrease in natural killer cell activity in the depressed children, but higher numbers of lymphocytes and B cells. The present experiment examined granulocyte function as measured by phagocytic and killing activity. While there were no overall effects of depression, there were striking age related effects such that the older children demonstrated increased phagocytosis (T = 2.4, p < 0.02) and killing (T = 2.2, p < 0.04). Family composition was shown to be significantly related to granulocytic killing, with those from non-intact families showing decreased phagocytosis (T = 2.4, p < 0.03). These data suggest that the stress of living in a non-intact family may be associated with altered phagocytic function.


GCRR in immune glands may be involved in hormone-modulated effects of stress on immune responsiveness. The effects of stress on the HPA axis are mediated by cortisol (C), and estradiol (E) and progesterone (P) receptor interactions are known to influence immune function. We have examined whether these gonadal hormones interact with the HPA axis to modulate immune function. We measured in the hippocampus, hypothalamus, pituitary, spleen, thymus and peripheral blood mononuclear cells. Type II receptor levels were higher in males than females, while Type I (mineralocorticoid) and Type II (glucocorticoid) receptors were measured in the hippocampus, hypothalamus, pituitary, spleen, thymus and peripheral blood mononuclear cells. Type II receptor levels were significantly higher in the hippocampus and spleen of SD, and in the thymus of L. Relative to SD rats, Fischer rats are hyper-responsive, and Lewis rats are hypo-responsive, to acute stress. The consequences of HPA axis differences between strains on habitation to chronic stress and on immune parameters are presently being investigated.

476.10 **CORTICOSTERONE RESPONSES TO INTERLEUKIN-1 AND RESTRAINT IN RATS LESIONED WITH 6-HYDROXYDOPAMINE**

H. Eduardo Chuluyan*, Adrián J. Dunn and David Saphier. Dept. of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, Louisiana 71130-3932

Stress and immune function are influenced by lesions in plasma concentrations of corticosterone (CS). To determine whether this phenomenon is affected by catecholaminergic dysfunction in neurons in the hypothalamic paraventricular nucleus (PVN), selective lesions were made, using 6-hydroxydopamine (6-OHDA). Injection of 6-OHDA into the PVN depleted noradrenergic fibers (NE) by 75%. Injection of the central noradrenergic ascending bundle depleted the hypothalamus of NE to a similar extent. This lesion also attenuated the CS response to glial cell line-derived neurotrophic factor (GDNF). The results indicate that the GDNF-induced increases in plasma CS concentrations depend at least partly on catecholaminergic activation of the hypothalamic-pituitary-adrenal axis associated with restraint.

[B]Supported by NS37283 and NH46261.
476.11 ALTERATIONS OF CENTRAL CATECHOLAMINES ASSOCIATED WITH PRIMARY AND SECONDARY IMMUNOLOGICAL CHALLENGE. S. Zakhern, N. Sharks, Z. Merali and H. Anisman. Departments of Psychology, Carleton Univ. and Univ. of Ottawa, Ottawa, Canada.

Central catecholamine variations in CD-1 mice were assessed after primary and secondary inoculation with sheep red blood cells (SRBC, 10^6 cells, ip). At the time of the peak primary and secondary immune responses (4 and 2 days post-inoculation, respectively), increased dorsal bundle norepinephrine (NE) utilization was evident. Moreover, like stressors, anagentic challenge provoked variations of mesocorticolimbic, but not nigrostriatal dopamine (DA). While the catecholamine changes associated with the primary response were only evident on the day of the peak response, the effects associated with the secondary response persisted for several days. Further studies using in vivo microdialysis in Sprague-Dawley rats confirmed the increased DA release in the nucleus accumbens associated with primary SRBC inoculation. However, this release occurred earlier than that detected in mice using HPLC determinations. These data are consistent with the proposition that the immune system acts as a sensory organ and that the immune response may be interpreted as a stressor leading to central catecholamine alterations.


Central nervous system activity may influence immune functioning, and conversely immune activation may affect central neurotransmitter activity. Administration of sheep red blood cells (SRBC) for instance, provokes brain region-specific alterations of norepinephrine and dopamine activity at a time which coincides with the peak immune response. The antigen-induced biochemical changes were reminiscent of those induced by stressors. The present investigation demonstrated that in CD-1 mice SRBC (10^6) inoculation, like stressor exposure, disrupted self-stimulation responding from the nucleus accumbens, coincident with the peak immune response. That is, self-stimulation was unaffected either immediately, 1 or 2 days after inoculation, but was markedly reduced 3 or 4 days after immunization. These data suggest a functional relationship between the immune system response and central neurotransmitter activity. Essentially, it is thought that immune responses are interpreted as stressors, ultimately leading to variations of central catecholamine activity, hence provoking an anhedonia.

476.13 δ̃ AND κ̃-, BUT NOT μ̃-, SELECTIVE OPIOID AGONISTS INHIBIT SYMPATHETIC POST-GANGLIONIC NEURON-DEPENDENT PLASMA EXTRAVASATION IN KNEE JOINT OF THE RAT.

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Plasma extravasation (PE) was induced in the knee joint of the rat by local infusion of either bradykinin (BK) (160 ng/ml), an inflammatory mediator produced at sites of tissue injury, or capsaicin (5 mg/ml). BK-induced PE is dependent on the sympathetic postganglionic neuron (SPGN), whereas capsaicin-induced PE is dependent on primary afferent neurons and the SPGN. When selective δ̃ (DPDPE) (10 μM), or κ̃ (U50,488H) (10μM) opioids were co-infused with BK-PE was significantly attenuated, and this attenuation was reversed by co-infusion of naloxone (1μM). In contrast, co-infusion of a selective μ̃-opioid agonist (DAMGO) (10 μM) did not reduce the PE induced by bradykinin. In bradykinin, δ̃ and κ̃-selective opioid agonists inhibit SPGN-dependent PE and μ̃-selective opioid agonist inhibits primary afferent but not SPGN-dependent PE. Supported by NIH grant AM42634

476.14 HEART RATE LEVEL, HEART RATE VARIABILITY, AND SPECIFIC ANTIBODY RESPONSE IN FREE-RANGING RHESUS MACAQUES.


The immune response may be influenced by a number of behavioral factors, mediated through two possible routes, circulating hormonal factors and direct autonomic innervation of lymphoid tissue. The present study related measures of autonomic reactivity to the appearance of tetanus toxoid (TT) specific IgM and IgG appearing in the plasma following TT immunization of juvenile Rhesus macaques free-ranging on the island of Cayo Santiago, P.R. Eleven subjects, 250-377 days old, were captured and briefly fitted with telemetry devices for unrestrained monitoring of EKG. A blood sample was obtained from each subject prior to immunization with TT on the day of release. After 10-15 days, subjects were recaptured and a second blood sample was obtained for the determination of plasma IgM and IgG specific to TT by an ELISA. A strong relationship between IgG levels and heart rate (r=.86, p=.003) and heart rate variability (r=-.79, p=.012) was noted. Plasma cortisol levels measured 24 hr following the first capture prior to immunization were not related to TT antibodies. (Supported in part by NIH Research Grant MH37373)

476.15 GENETIC DIFFERENCES IN SOCIAL BEHAVIOR: RELATION TO CANCER SUSCEPTIBILITY AND NK CELL FUNCTION.


Much of the literature concerning social behavior has focused on understanding how various social and non-social "states" can influence aspects of immune function. Despite considerable evidence indicating the relationship between personality variables and disease susceptibility (e.g., cancer), few studies have related immune function to genetically-determined sex differences that are stable across development ("trait"). We studied the 23rd generation (G23) of ICR mice selectively bred for high and low levels of aggression. The high aggressive line (NC900) shows rapid and low levels of aggression. The low aggressive line (NC100) has departed substantially from the high line for aggression (G23), exhibiting high levels of freezing ("fear") in response to social contact. This robust and reliable line difference appears to be correlated with differences in midbrain dopamine function. We hypothesized that the genetic differences in social behavior observed in NC100 mice would correlate with increased vulnerability to cancer development and reduced natural killer (NK) cell function. To test this, 3-methylcholanthrene-induced tumor development and splenic NK cell activity in the NC900 and NC100 lines. Only 44% of the NC900 mice developed tumors, whereas all NC100 mice had palpable neoplasms. Additionally, baseline NK activity was significantly lower in socially isolated (NC100) mice versus aggressive (NC900) mice. Conversely, no baseline differences in NK activity were found between NC900 and non-selected, socially isolated mice (NC 600). These latter data complement existing behavioral evidence supporting the unidirectional nature of the selective breeding program. Our data support the hypothesis that genetic ("trait") differences in social behavior may predict differences in disease susceptibility and immune function. Integration of future neurobehavioral and immunological findings using this mouse model may provide important information concerning mechanisms of brain-immune interactions. (Supported by the Foundation of Hope and MH45371)

476.16 BEHAVIORAL DIFFERENCES IN LEW/N AND F344/N RATS.


Recently several differences in the neuroendocrine response to inflammatory mediators have been characterized between F344/N and LEW/N rats. These differences are important because they are related with the differential susceptibility to arthritis across these strains. Previous studies have indicated that this susceptibility is mediated by a deficiency in central corticotropin releasing hormone (CRH) biosynthesis or secretion, and is reflected by a difference in hypothalamic-pituitary-adrenal response during stress in these strains. We have also shown that the LEW/N rat hyperteresponds to acoustic startle stimuli whereas the F344/N rat hyporesponds, compared to Sprague-Dawley rats. Contrary to previous studies using environmental stressors, the F344/N rats exhibit an exaggerated corticosterone response to startle stimuli while the LEW/N rats do not. The current studies sought to further characterize potential behavioral differences in these strains. LEW/N rats consistently ambulate less than F344/N rats in an open field, with the ratio of time spent in inner, as opposed to outer, portions of the field being approximately 0.1 for the LEW/N and 0.4 for the F344/N rats. CRH decreased ambulation in both strains, but did not alter this ratio in either strain. In contrast, LEW/N rats entered the open arms of an elevated plus maze more often than F344/N rats, but the time spent per entry was the same for both strains. These results demonstrate that these strains differ in baseline response on several behavioral tests. These different responses mediated by various environmental influences, substantiating the usefulness of behavioral markers for neuromedimmune disease.
**447.17** BEHAVIORAL EFFECTS OF REPEATED INJECTION OF INTERFERON-α OR INTERFERON-β ON REWARDING ACTIVITY AND PRODUCTION OF LIVER P-450 DEPRESSION IN MICE (RENTON ET AL., 1984) BUT BEHAVIORAL EFFECTS ARE UNKNOWN. MEASURES OF ACTIVITY (OPEN FIELD), DEPRESSION (TAIL SLAM), MALE STRENGTH (FORELIP GRIP STRENGTH) AND MOBILITY (SWIM) AND ENDURANCE WITH 0.3, 3, AND 5 X ADDED BODY WEIGHT WERE EXAMINED IN MICE CΕ MICE EXPOSED TO 1600 U/G OF IFN-α/β DAILY FOR 5 DAYS (N = 9) OR TO THE IFN VEHICLE (N = 11). BEHAVIOR WAS TESTED ON EACH DAY, EXCEPT FOR SWIMMING WHICH WAS TARTED ON DAY FIVE.

Open field activity was significantly depressed on all days of testing in the group exposed to IFN (p < 0.02). The number of the times the head dipped under the water when swimming with 3X added body weight was higher in the IFN treated mice (24.1 vs 8.9, p < 0.025). Thus, this human hybrid IFN is behaviorally active in mice. Depression of motor activity is a general effect as seen both in this study and in our prior study with acute doses of non-recombinant mouse IFN-α (Lee et al., 1980). The generalizability of the effect is seen across different activity measures in these two studies. Further, swim endurance is depressed by IFN. Supported by grants MO00621, MO00624, and MO14442.

**447.19** CLINICAL AND IMMUNOLOGICAL CORRELATES OF ANTI-HIPPOCAMPAL IGG ANTIBODIES IN SCHIZOPHRENIA.

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Raised hippocampal autoantibody levels may occur in a subgroup of schizophrenic patients characterized by negative symptoms, low IL-2 production and the presence of non-CNS autoantibodies. Using an enzyme immunoassay (ELISA), we tested for IGG autoantibodies to human hippocampus in 99 RDC schizophrenic patients and 81 normal controls matched for age and the presence of autoantibodies to non-CNS antigens. Anti-hippocampal antibody levels were correlated with the severity of positive and negative symptoms and disease chronicity (r = 0.25, P < 0.01; Interleukin-2 (IL-2) production). First episode subjects had lower hippocampal autoantibody concentrations than patients (P = 0.01). Acutely ill patients with non-CNS autoantibodies had higher anti-hippocampal antibody levels (P < 0.02). Negative symptoms were significantly correlated with anti-hippocampal antibody levels in remitted patients (r = 0.44, P = 0.016). Patients with anti-hippocampal autoantibody levels above the 75th percentile had the lowest IL-2 production (P = 0.029).

**447.21** ANOREXIC EFFECTS OF INTERLEUKIN-1 (IL-1) ARE MEDIATED PERIPHERALLY.

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IL-1 is a cytokine that is released by activated macrophages and monocytes and mediates many of the local and systemic responses to inflammation. Although both peripheral and central injections of IL-1 produce anorexia, it isn't clear whether this effect is mediated peripherally, centrally, or both. Recently, a specific antagonist for the IL-1 type 1 receptor has been characterized and cloned (IL-1ra; Synergen, Boulder, CO). We have used this protein to determine the site of action for the effects of IL-1 on feeding. Male rats were pair fed and restricted and trained on an operant schedule (FR10) for food reinforcement. Rats were tested for 5 min before injection of recombinant human IL-1β (Glaxo, Geneva; 4 μg ip or 40 ng iv) and then tested 1, 2, 4, 8, and 24 h later. Both ip and iv injections produced profound decreases in responding, with maximal effects 2-4 h post-injection. IL-1ra pretreatment (8 mg/kg ip or 80 μg/kg iv) completely blocked these effects when administered by the same route. In contrast, iv IL-1ra was unable to block the anorexic effects of ip IL-1. IL-1ra had no effects by itself, these results are in agreement with previous suggestions that the anorexic effects of IL-1 are mediated peripherally.

(Supported by DRET (RD) and ONR (KKW))

**447.18** SECOND-ORDER AND SENSORY PRECONDITIONING OF IMMUNOSUPPRESSION: EVIDENCE FOR COGNITIVE CONTROL.


Two experiments evaluated the effects of second-order conditioning on immune function. In the first, rats were given pairings of stimulus A and shock and then pairings of stimulus B and A but without shock. Control subjects received the same except that B and A were unpaired. After a recovery period, different groups received exposure to A, the paired B (B+), the unpaired B (B-), the training context (X), or no treatment (X) just before sacrifice. Mitogen-stimulation assays of lymphocytes from the spleen and blood and an MNC cell assay of splenocytes showed comparably pronounced immune suppression for the A and B+ groups, relative to the B-, X, and N groups, attesting to a second-order effect for B+.

These results suggest that immune function is affected by psychological forms of stress that derive from a cognitive processing of events; i.e., B+] implies A and A implies shock. To corroborate this, we conducted a second experiment on sensory precon- ditioning. That study duplicated the first except that pairings (or unpairings) of B and A occurred prior to pairings of A and shock, so as to preclude association of B+ and A's suppressive effect. In evidence of cognitive control, the results showed comparably pronounced immune suppression for the A and B+ groups, relative to the rest, establishing that "worrisome" thoughts about something, even remote, profoundly affect immune function.
477.1 DIFFERENT PROJECTIONS FROM THE LATERAL PRAEBRACHIAL NUCLEUS AS DEMONSTRATED BY PHA-L, RN-Slugg and AR Light. Dept. of Physiology, School of Medicine, Chapel Hill, NC 27599.

To determine the projection sites of nociceptive and thermoreceptive neurons, discrete injections of PHA-L were made into the PBN in a series of experiments which included unilateral recordings from single cells. We were able to demonstrate that the PBN projects to the anterior cingulate cortex, the posterior thalamic nucleus, the central medial thalamic nucleus, and the ventrolateral thalamic nucleus. The results suggest that the PBN plays a role in the mediation of pain-related responses.

477.2 DISTRIBUTION OF NEUROTRANSMITTERS IN THE CAT'S BRAINSTEM AND THALAMUS. D. Harde, C. Vahl-Knöffe. Physiological Institute, Universität Würzburg, D-8700 Würzburg, Germany.

To further characterize the distribution of neurotransmitters in the cat, we have studied the distribution of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), in the brainstem and thalamic nuclei. We have used immunohistochemical techniques to label GABA-containing neurons and we have found that GABA is distributed throughout the brainstem and thalamic nuclei, with highest densities in the ventrobasal thalamic nucleus and the posterior thalamic nucleus. The results suggest that GABA plays a role in the mediation of pain-related responses.
ULTRASTRUCTURE OF LAMINA I TERMINALS IN NUCLEUS CENTRALIS OF THE CAT. A. Blomqvist, A.-C. Ericson*, J. Broman and A.D. Craig. Dept of Cell Biology, University of Linköping, S-581 83 Linköping, Sweden, and Division of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013, U.S.A. Previous anatomical studies have demonstrated that the release of substance P by lamina I terminals in the spinal dorsal horn seems to be a specificity of the specific lamina I terminal site in the medial thalamus. In the present study, the ultrastructure and synaptic organization of the lamina I terminals in Sm were examined. Of our 198 retrogradely labelled cell bodies (RPHal) were injected into lamina I at different spinal levels. PHAL-containing axon terminals in Sm were demonstrated with the peroxidase-antiperoxidase method and observed in the electron microscope. Labeled terminal boutons packed with round synaptic vesicles were seen in synaptic contact with dendritic profiles that were presumably neurites from laminal macroreceptive relay cells. Synaptic contacts were also seen with presynaptic dendrites that in turn synapsed with relay cell dendrites. Examination of serial sections suggests that such synaptic contacts exist both independently and in the triadic arrangement typical of thalamic sensory relay nuclei. Complex synaptic aggregations in which a relay cell dendrite received inputs from several lamina I afferent terminals and presynaptic dendrites were found. These results suggest that processing of lamina I afferent inputs in Sm is different, probably via GABAergic interneurons. Supported by grants from the NIH and the Swedish Medical Research Council.

NOXIOUS HEAT AND INNOCUOUS COLD PERCEPTION IN MONKEY AFTER INJECTION OF LIDOCAINE INTO THE VPM THALAMIC NUCLEUS. M.C. Bushnell, J.L. Oliva, N. Baxt, D. Tramby and G.J. Dugas. Fac. Med., Univ. Quebec, HEC, Quebec, Canada, H3C 3T7. In thalamus, information about pain and innocuous cold is transmitted to the ventroposterior nuclei (VPM and VPM) and to more medial structures, such as the subnucleus. The present study evaluates the role of VPM in facial pain and temperature perception, by determining the effect of transient inactivation of VPM on a monkey's detection of changes in intensity of noxious heat and innocuous cold. Two chloralose monkeys were trained to detect small differences in the intensity of a white light and of noxious heat (near 46°C) and innocuous cold (near 30°C). Thermal detection thresholds were determined using the method of limits. Two chloralose monkeys were trained to detect small differences in the intensity of a white light and of noxious heat (near 46°C) and innocuous cold (near 30°C). Thermal detection thresholds were determined using the method of limits. These results suggest that processing of nociceptive and innocuous inputs in Sm is different, probably via GABAergic interneurons. Supported by grants from the NIH and the Swedish Medical Research Council.

THE TRIGEMINO-PONTO-AMYGDALIAN PATHWAY DEMONSTRATED BY RETROGRADE TRANSENSURAL TRANSPORT OF PSEUDOPEROXIDASE. L.J. Leonard, J.C. Card and A.L. Baughman, Dept. Anatomy and Physiology, UCSP, San Francisco, CA 94143 and DuPont-Merck, Wilmington, DE 19898. Although limbic structures must contribute to the affective component of the pain response, the circuitry through which nociceptive inputs access forebrain sites is poorly understood. Recently, Bernard et al. (1989) used a combined amino acid and retrograde tracer study that included several dopaminergic and noradrenergic structures and found that the transmission of nociceptive messages. We have initiated a parallel series of studies using an alpha-hexadecylphosphorylcholine (AHP) as the retrograde tracer to evaluate the afferent projections of the spinal cord and the thalamus. We injected AHP into the spinal cord and thalamus and directly or indirectly access several forebrain sites implicated in pain and thermal nociception and observed these terminals in the spinal cord and thalamus. AHP injection into the spinal cord and thalamus were confirmed to block the activity of neurons recorded 2mm from the injection site, and produced no decrement in the monkey's detection ability. Three similar injections of AHP in the spinal cord and thalamus were made. Single cell recordings identified regions within VPM and VPL and VPM that were histologically verified to be in Sm. Most of these neurons could be driven by Aδ and C fibers. The excited as well as the inhibited neurons in Sm were depressed by systemic morphine in a dose-related and naloxone-reversible fashion. Thus these results support the hypothesis that Sm may be involved in mediating the affective-motivational aspects of pain. (Supported by NIMH)

NOXIOUS TEMERAL, CHEMICAL AND ELECTRICAL STIMULATION. J.-M. Robert, C.Y. Chiang and J.-G. Doostparvar. Dept. of Physiology, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada. Recent studies have suggested that Sm may be an important thalamic relay for nociceptive information. The present study investigated the effects of thermal, chemical and electrical stimulation of Sm neurons in urethane/chloralose anesthetized rats. Nociceptors but no innocuous mechanical stimulation induced responses in 67 of the 86 neurons studied that were histologically verified to be in Sm. Most of these neurons were also activated by noxious heating (mean threshold 47.6 ± 0.4°C), but in many cases the responses to increasing temperatures were not well graded. Many of these neurons were also excited or their response to noxious stimulation facilitated by subcutaneous, intramuscular or intraperitoneal injection of 2.5% formalin or hypertonic saline (0-1.2 ml). Thirty-eight of 49 Sm neurons tested could be activated by intense electrical stimulation (15-25ms 200 Hz trains, 3 m pulse duration, 5-10 mA/3 s) of tail, paw and vibrissal pad. The latency of the responses ranged from 20 to 500 ms (mean 174±27 ms, SEM). The noxious evoked responses of the primary afferents eliciting the responses were estimated for cells by comparing the latencies of the Sm neuron responses to stimulation of the tail at distal and proximal sites and ranged from 10 to 150ms. The Spinothalamic and Pseudoplateauak to electrical stimulation often appeared only after repetitive train stimulation, and then showed "wind-up", suggesting a polysynaptic pathway to Sm. The response characteristics of the Sm neurons in this study support the hypothesis that Sm may be involved in mediating the affective-motivational aspects of pain. (Supported by NIMH)

SPINO-THALAMIC (STT) ACTIVATION OF PRIMATE THALAMIC NEURONS IS NOT MODULATED BY GABAERGIC LOCAL CIRCUIT SYNAPSES. H.J. Ralston, III, A.M. Milroy and D.D. Ralston, Department of Anatomy, University of California, San Diego, California, 92103. We have examined several hundred labeled STT terminals in macaque monkeys and have contrasted their synaptic characteristics by labeled medium (ML) and large (LL) terminals. Anesthetized monkeys received microinjections of 10-15 nl of wheatgerl agglutinin horseradish peroxidase (WGA-HRP) into the dorsal horn of the cervical or lumbar spinal cord. Following injection of HRP, WGA-HRP were visualized and numerous mitochondria. ML terminals contact dendritic shafts of projection neurons and, in more than 95% of cases, the GABAergic appendages of local circuit neurons (LCN) which have contact the projection dendrites to form a triadic relationship and presumably mediate feed forward inhibition. In contrast, STT terminals form axo-axonic synapses with projection neurons are rarely subject to GABAergic modulation by thalamic LCN's. Supported by NS 21445 from the N.I.H.

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477.7

477.8
477.13 IS THERE A NOCICEPTIVE AREA IN N II ? EVIDENCE FROM LASER EVOKED POTENTIALS IN HUMANS.

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In a previous study (EEG J 70, 1988, 429-441), we found a mid-latency component of the laser evoked potential that projected to the somatosensory cortex. We now describe the scalp topography of this N1 in detail and distinguish it from the vertex potential (N2).

CO2-laser beam (15 W, 20 ms, 3 mm diameter) was applied to the dorsum of the left hand in 15 subjects. The EEG was recorded from 15 leads, distributed over the whole scalp (bandpass 0.33-200 Hz, linked earlobes reference).

The laser evoked potential began at 140 ms after stimulus onset, with a negativity in contralateral posterior temporal leads (N1). At 170 ms it was significantly larger than ipsilateral to the stimulus (T4 vs. T3). Simultaneously with this posterior temporal negativity, a significant positivity appeared in frontal midline leads. The vertex potential began at about 200 ms and reached its maximum scalp negativity (N2) at 240 ms in the lead Fz. There was no concurrent localized positivity.

The N1 may reflect activity of a tangential generator in or near SII. After electrical nerve stimulation (radial or median nerve) no potential with such a lateral localization could be identified. The N2 topography corresponds to that of other vertex potentials.


Partial sciatric ligation in rat produces a peripheral neuropathy associated with hyperalgesia and allodynia similar to that seen in human neuropathy. The mechanism by which ligature produces these effects is unknown. Based on evidence that excitatory amino acids play a role in noceception at the spinal cord level and that their release is modulated by the nociceptive neurotransmitter substance P (SP), we examined whether ligation affects amino acid release in the spinal cord in response to chemical nociceptive stimulation or SP.

Partial sciatric ligature or sham surgery of male rats was done using the method of Bennett and Xie. Seven days later, loop dialysis and infusion cannulae were implanted in the lumbar intrathecal space. The CIFT of animals was dialyzed and 2 samples were collected before and after the intrathecal injection of 2.5 nmoles of SP.

Mustard oil produced a significant release of both Asp, Glu and Tau, however, these results are consistent with the hypothesis that the neuropathy associated with sciatric ligation and sham operated animals. Likewise, the magnitude of the SP-induced release of Glu or Tau was also not altered by ligation. In contrast, SP-induced release of Asp was significantly enhanced by ligation.

These results are consistent with the hypothesis that the neuropathy associated with sciatric ligation is mediated through SP-induced Asp release. This enhanced release of Asp may reflect an up-regulation of SP receptors secondary to a decreased release of SP from primary afferents. Chemospecific pain-mediated release of excitatory amino acids is not significantly changed in peripheral neuropathies. Supported by USPHS Grants DA04090, DA01024, DA04190, DA07234 and CA01342.

478.3 MEDIATION OF KAINATE-INDUCED BEHAVIORAL SENSITIZATION BY SUBSTANCE P (SP) AND SP N-TERMINAL FRAGMENTS IN THE MOUSE.

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Sensitization to the behavioral effects of repeated intrathecal (i.t) injections of kainic acid (KA) in mice is inhibited by pretreatment with 0.8 μg of capsaicin i.t. The present study was designed to characterize the interaction between KA and SP in vivo. Pretreatment of mice at two min intervals with three injections of 7.5 μg each of KA or SP (10 pmol) produced the behavioral response to subsequent injections of KA, while pretreatment with SP (75 μg) or KA (25 μg) alone did not alter the intensity of responses to KA. This suggests that the N-terminal rather than the C-terminal of SP enhances subsequent KA-activity. Sensitization to the N-terminal fragment of SP was not reversed by pretreatment with capsaicin.

These data show that alteration of SP receptor activity in the dorsal horn using a method of SP receptor desensitization does not affect tonic nociceptive behaviors. These data suggest that SP in the dorsal horn does not mediate tonic nociception. Supported by the MRC Research Institute.

478.4 EXAMINATION OF THE ROLE OF SPINAL SUBSTANCE P IN TONIC NOCICEPTIVE BEHAVIORS.

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Substance P (SP) in the dorsal horn has been shown to mediate certain types of phasic noceception. Intrathecal administration of SP reduces tail-flick and paw pressure latency in the rat. However, these responses were attenuated following successive administration of SP. We have confirmed this observation and determined that behaviors remain attenuated for up to 150 minutes and that these behaviors correlate with a reduction in the number of SP binding sites and a decrease in SP binding affinity in the dorsal horn. In this study, we examined the role of SP in tonic spinal nociception using a method of SP receptor desensitization. Two models were employed: the formalin test and the monosodium urate (MSU) test. Male Wistar rats (300-400g) were implanted with intrathecal catheters. SP (0.7nmol, i.t) was administered at 0, 30 and 60 minutes. Rats were then injected into the hindpaw with 5% formalin (100 μl) into the ankle-joint with MSU (1 mg dissolved in 0.25 ml 10% Tween 80). Formalin and MSU-induced responses were assessed by measurement of autotomized behaviors and hindpaw pressure, respectively.

In the formalin test, the repeated administration of SP reduced the first phase of pain-related behaviors but did not affect the second phase of pain-related behaviors. In the MSU model, the repeated administration of SP did not have any effect on MSU-induced pain-related behaviors.

These data show that alteration of SP receptor activity in the dorsal horn using a method of SP receptor desensitization does not affect tonic nociceptive behaviors. These data suggest that SP in the dorsal horn does not mediate tonic nociception. Supported by the MRC Research Institute.

Exciatory amino acids and substance P are involved in sensory afferent transduction of nociception and in spinal processing of nociceptive information. We have studied the spinal actions of substance P in the formalin test in mice. Formalin (1%) was injected in a volume of 20 µl. Biting or shaking of the injected paw was scored. The substances were administrated intracerebrally in a volume of 7 µl. A significant increase of the responses in the late phase of the formalin test (20-40 min after injection of formalin) was observed after intraarehal coadministration of NMDA (0.125 nmol) and substance P (2 pmol).

Single administration of either NMDA or substance P did not induce any change in the response. The combined activation of NMDA receptors and substance P receptors induces a sensitization of the nociceptive response in the formalin test.


The functional interaction between substance P and excitatory amino acid (EAA) agonists in the spinal cord was studied. All agonists were injected intraarebrally in a volume of 7 µl. A strong potentiation of the behavioral response (biting and scratching) was observed when substance P (2.5 ng) was coadministered with NMDA (0.025 nmol), AMPA (0.0125 nmol) or kainic acid (0.025 nmol). The effect of each drug as well as of the combinations was blocked by the corresponding antagonists injected 5 min prior to the agonists: the selective NMDA receptor antagonist CPP (1 mmol), the non-NMDA receptor antagonist CNQX (2 mmol) and the substance P analog Spantide I (5 µg).

These findings indicate a functional interaction between substance P and EAA neurotransmitters in the spinal cord, compatible with the hypothesis that corelease of these substances from primary afferent neurons may enhance nociception.
MK 801, AN NMDA RECEPTOR ANTAGONIST, POTENTLY REDUCES NOCICEPTIVE BEHAVIORS IN RATS WITH PERIPHERAL MONONEUROPATHY. A. Vaccarino, W. Liebeskind, J. Maier, D. Magee. Dept. of Neurosurgery, Physiology, and Anesthesiology, Medical College of Virginia, Richmond, Virginia 23298

Hypersensitivity in neuropathic pain syndromes could result from either abnormal peripheral nociceptive input or overactivity of endogenous excitatory amino acids at primary afferent synapses. In the present experiment, we examined the effect of MK 801, a non-competitive NMDA receptor antagonist, and a local anesthetic agent, bupivacaine, on thermal hyperalgesia associated with a rodent peripheral mononeuropathy produced by sciatic nerve ligation. Intrathecal injection of MK 801 or vehicle (5 ml) was administered 1 hour prior to 1 day of hindpaw mechanical hyperalgesia. Intrathecal bupivacaine was administered 1 hour prior to 1 day of hindpaw hyperalgesia. Intrathecal hyperalgesia was assessed 24 hrs after injection and on days 5, 6, and 7 postinjury. Hyperalgesia to radiant heat was significantly reduced 24 hrs after injection in animals receiving either MK 801 (10 nmol) or saline (10 nmol) at the lumbar spinal cord, or perivascular injection of 0.5% bupivacaine (0.6 ml), but not in rats receiving saline or MK 801 (10 nmol) at spinal cord thoracic segments (ANOVA, P < 0.001). Active avoidance behavior was measured for 30-90 min. Flexion of rats pretreated with MK 801 was significantly reduced (95% above rats that had received saline (X = 7.3g, sem = 1.0g). Rats that were returned to individual cages for recovery. At 3 days, rats were reassessed and flexion was measured again. Rats that had received MK 801 showed a mean flexion of 16.2g (X = 1.7g), 110% above rats that had received saline (X = 7.3g, sem = 1.0g). Rats were then spinalized and flexion was measured again. Rats that received saline were given a single injection of 50 mg/kg of MK-801 intrathecally 3 days prior to surgery. Rats that had received MK-801 were given saline instead. Supported by Fidia Pharmaceuticals.

MK 801 BLOCKS THE INDUCTION OF PERSISTENT HINDLIMB FLEXION DUE TO MUSCLE STIMULATION IN INTACT AND SPINALIZED RATS. M. F. Anderson and B. J. Winterson, Department of Physiology, University of New England, College of Osteopathic Medicine, Biddeford, ME 04005.

We have shown that NMDA antagonists interfere with the induction of peripherally-induced persistent hindlimb flexion (Anderson & Winterson, Neurosci. Abstr., 1988) and that muscle stimulation alone is sufficient to produce persistent flexion comparable to that generated by percutaneous stimulation (Anderson & Winterson, Neurosci. Abstr., 1990). The present experiments examine whether NMDA receptor blockade during muscle stimulation attenuates persistent hindlimb flexion.

In pentobarbital anesthetized adult Long-Evans rats, skin was removed from the thorax and hindlimbs, exposed muscle was wrapped in saline moistened gauze. Rats were kept warm via a thermostatically controlled blanket. Wound clips were applied to the muscle through the gauze, current (2mA, 7mA, 10Hz, 1 hr) was delivered across the wound clips. Twelve rats were stimulated intact (CONTROL) and 12 rats were spinalized prior to skin removal (SPINAL). From each group, half were treated with the non-competitive NMDA receptor antagonist, MK-801 (10 mg/kg, i.p.) 1 hr prior to stimulation (MK-801) and SPINAL-MK-801). Following stimulation, lidocaine (12.5 mg/kg, i.m.) was injected at the wound clip site. Flexion was measured 30-90 min. Flexion of rats pretreated with MK-801 was significantly less than rats that had received saline (X = 7.3g, sem = 1.0g). Rats were then spinalized and flexion was measured again. Rats that had received MK-801 showed a mean flexion of 16.2g (X = 1.7g). These data suggest that NMDA receptor activation may be critical to chronic neuropathic pain syndromes, and the pharmacological intervention by excitatory amino acid antagonists may be an important approach for clinical treatment of neuropathic pain.

SWIM STRESS PRODUCED ANALGESIA IN THE FORMALIN TEST IS MEDIATED VIA THE NMDA RECEPTOR. A. L. Vaccarino, P. Marek, W. Sterlberg and J. Liebeskind, Department of Psychology, University of California, Los Angeles, CA 90024.

Different forms of stress can produce analgesia. In the hot-plate test, a 3-minute swim stress of 32°C produces analgesia which is predominately opioid in nature. In contrast, the analgesia produced by a 20°C swim stress has a strong non-opioid component which is centrally mediated and is blocked by the N-methyl-D-aspartate (NMDA) antagonist, MK-801. To determine the effect of swim stress on C-fiber responses, we used the formalin test. Male Swiss mice (30-40 g) were injected subcutaneously with nocaoline HCl (1 mg/kg), MK-801 (0.075 mg/kg), or saline 15 minutes prior to swimming for 3 minutes in water maintained at 20°C or 32°C. An additional group of mice received saline. Following a 2-minute drying off period, the mice were injected with 20 pl of 5% formalin into the plantar surface of one hindpaw. The animal’s pain-related behaviour (time spent licking the injected paw) was then continuously rated during the subsequent 10 minutes. Swim stress produced a significant suppression of formalin pain scores relative to non-stressed controls at both the 20°C and 32°C C-fiber temperatures. No significant differences were found in the magnitude of analgesia produced at these two temperatures. The analgesia produced in both the 20°C and 32°C swim stress was completely abolished in mice pre-treated with MK-801. These data suggest that the analgesia produced by swim stress in the formalin test is non-opioid in nature and is mediated at the NMDA receptor. Supported by an unrestricted grant from the Bristol-Myers Squibb Company and NIH grant NS 07628.
MK-801 ATTENUATES NON-OPIOID STRESS-INDUCED ALGESIA IN SELECTIVELY BRED MICE: COMPARISON ACROSS THREE SWISS STRESS PARAGRAPHS

479.4

THE EFFECT OF 40 Hz FLICKER ON THE PERCEPTION OF GLOBAL STIMULUS PROPERTIES

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Neurons in the cat visual cortex show oscillatory responses in the range 20-60 Hz. Under certain conditions, influenced by global stimulus properties, neurons in spatially separate functional columns synchronize their oscillatory responses. This has been interpreted as support for the hypothesis that synchronized oscillatory responses serve to establish relations between spatially separate features. If these coherent oscillations influence perceptual judgments, we reasoned that neuronal oscillations induced by flickering visual targets might have an effect on visual perception. We measured psychophysical performance of human subjects in a texture segregation task, where the texture elements flickered against a gray background at a temporal frequency between 20 and 50 Hz. The stimuli were fields containing line segments whose position was jittered from a regular array. A rectangular patch contained segments differing in orientation from the rest of the field. The subject's task was to indicate whether the boundary of the rectangular patch was orientated horizontally or vertically. We varied the difference in orientation between the segments inside and outside the rectangle to find a value that would maximize performance. We found four temporal phase conditions: no flicker, synchronized flickered of all texture elements, synchronized flicker of the elements within one region, and unsynchronized flicker of all texture elements.

Performance depends in the expected way on signal strength as determined by contrast. If, however, entirely independent of the temporal pattern of the flicker. We conclude that either stimulus-induced oscillations do not interfere with other oscillatory responses, or that the physiologically observed oscillatory responses are not necessary for perceptual grouping.

479.3

SYNCHRONIZED OSCILLATIONS DURING COOPERATIVE FEATURE LINKING IN A MODEL OF VISUAL CORTEX

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A model of synchronized oscillations in visual cortex is presented to account for neurophysiological findings (Eckhorn, et al, Biol. Cyber., 60, 1988, Gray, et al, Biol. Cyber., 23, 1976:1978) which predicted that cortical oscillations would be expressed by resonance standing waves in which cooperatively linked cells oscillate in phase with one another. These results suggest how research on this topic can be integrated into formal models of visual perception in cortex.
479.5 CHANGES IN GAMMA-BAND ACTIVITY OF RHESUS MACAQUE NEOCORTEX DURING A VISUAL PATTERN DISCRIMINATION TASK. S.L. Broder, Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431 and NIMH, Bethesda, MD 20892.

Recent findings of stimulus-dependent gamma-band (25-75 Hz) activity in visual cortex have led to the proposal that this frequency band plays a functional role in cortical processing. Transcranial field potentials were simultaneously recorded from 16 chronically implanted bipolar electrodes in rhesus macaque monkeys as they performed a visual pattern discrimination task. The electrodes were distributed across the convexity of the hemisphere contralateral to the preferred hand. The monkeys were trained to respond to one stimulus pattern type (4 squares arranged in a diagonal line) by lifting a lever within 500 msec (GO task), and to another type (the same squares arranged in a diamond) by holding the lever for 500 msec (NO-GO task). The gamma band was isolated by digital filtering, and the rms amplitude was computed for each of 16 100-msec-long windows spanning 700 msec post-stimulus. For each task type, the rms amplitude of each window was averaged over all correctly performed trials. The averaged gamma amplitude showed location-specific deviations from baseline. The magnitude and timing of these deviations differed according to task type, the strongest changes occurring before 500 msec in the GO task and after 500 msec in the NO-GO task. These findings suggest that activity in the gamma frequency band in many cortical regions may have a functional role in performance of visual pattern discrimination tasks. (Supported by NIMH Grant MH43730 to EEG Systems Laboratory, San Francisco, CA.)

479.6 SPATIO-TEMPORAL DYNAMICS OF STIMULUS-INDUCED VISUAL ATTENTION. S.Miyaih*** and S.Shimojo**.

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We have shown that the focus of visual attention is detected by motion sensation in an adjacent line (Hikosaka et al., ARVO'91). When a spot (cue) appears on a screen, followed by appearance of a line, the line is perceived to be drawn from the cue side. This is because the cue facilitates the perception of the nearest portion of the line (Kawachi et al, Vision Res). We now report that the spatio-temporal dynamics of attention can be plotted using a short line (<0.2deg) as the probe. While the observer fixated on a spot, a cue spot and then a short line appeared. We found that the motion sensation was greater when the line was closer to the cue stimulus. We used a cancellation method to examine the spatio-temporal dynamics of attention. When the probe line was drawn in the direction toward the cue so as to cancel the attention-induced motion effect. If the attentional effect was large, we needed to draw the line more slowly. The speed of the line drawing was taken to be a measure of local attention gradient. A two dimensional map of the magnitude of attention was thus obtained. We studied the effects of single and multiple cues. A single cue produced a single peak with quasi-exponential decay outward. The map changed its magnitude and shape after onset of the cue stimulus. Two cue stimuli produced two corresponding peaks, suggesting the presence of multiple focci of stimulus-induced attention. We also studied the spatial and temporal interactions of the two stimuli.


A 51 year old patient suffered bilateral strokes involving the right occipito-temporal cortex. He presented with a bilateral superior altitudinal field defect, with normal fundoscopy and ERG.

In addition to his achronomatopsia (reported by Hagestrom-Portnow, et al, ARVO 1991, p1215), he had severe agnosia, particularly with more complex visual forms such as faces, and he could read very difficult material, albeit haltingly. Surface representation seemed largely intact toposensorial as seen by岛田. The patient demonstrates Kanizsa figures demonstrating attentional blindsight.


Theories of attention postulate a mechanism that shifts attention in space and is organized in direction and/or field specific coordinates. To examine the idea, normal subjects were scanned with PET activation methodology during a task in which visual field (left, right) and direction of attention movements (left,right) were manipulated in a blocked design. Previously we have reported a superior parietal activation dependent on the field of attention shift. The task display was a horizontal row of small boxes extending into both hemifields. Subjects detected a star presented for 150 ms within one of the boxes. On 80% of trials the order of probable locations predictably followed a particular direction; on 20% random positions were probed (shifting task). A central detection task was run in which peripheral probes were presented but subjects detected the onset of a star in a central box. PET sessions also included a fixation point control with no stimulation as well as a passing control in which the peripheral stimuli were presented but no task performance was required. PET subtraction images were obtained using shifting, central detection-, and passive-fixation conditions. These images indicated that: 1. A region of the prefrontal cortex was activated when subjects shifted attention in the contralateral visual field, but not during central detection, with minimal activation when the stimuli were passively presented; 2. There was no evidence of activation related to attention shifts in a particular direction. This is similar to the results found in the superior parietal lobe, except that parietal activation was seen in the passive condition. These results are consistent with an interpretation that both parietal and frontal mechanisms contribute to shifting attention in space, but serve different task demands.

479.9 ACTION-BASED SPATIAL ATTENTION IN NORMAL AND HIPPOCAMPAL LESIONED MONKEYS. Michael Taffe*, Beth O. Moore, Steven P. Tipper* and Gordon C. Breith, Dept. of Psychology, U. C. San Diego, CA 92093, and Dept. of Psychology, McMaster University (S.P.T.).

Human subjects display an action-based attentional system when tested on a task that requires them to reach for a target stimulus. Distractor objects on the action path towards the target cause much more interference than those located below the target (Tipper et al., Psychonomic Soc. Meeting, 1990). In order to prevent inadvertent responses to distracting stimuli on the path to the target, it is hypothesized that the distractors use a mechanism of distractor inhibition. We examine the extent to which spatial attention in macaque monkeys is organized in a similar way.

It was found that a spatial pattern of correct responses, both normal and hippocampal-lesioned monkeys was very similar to that seen in normal humans. It is suggested that inhibition of distractor items is a mechanism also utilized in infra-human primates. Hippocampal lesions were found not to affect performance of this task in any way. This suggests that while lesions to the hippocampal system may impair monkeys' ability to make spatially selective responses (Rupniak & Gaffan, J. Neurosci., 7, 2331, 1987), spatial attention remains largely intact.

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479.10 VISUAL SPATIAL ATTENTION DEFICIT IN DYSEXICS. EVIDENCE OBTAINED FOR COMPUTER PROGRAMS. Zarco de Coronado L., Gutierrez L.A.* and Orozco C.* Dpto. de Fisiologia. Fac de Medicina, C.P. 70250 and Ito. Nal. de la Comunicacion Humana. C.P. 01480, Mexico.

Computer animated programs were prepared to be applied in normal and dyslexic children. The exercises started with a sample figures, letters, numbers or special lines patterns with 1-4 discrimination details. They are followed by 10 fixed or 24 randomized complete or incomplete test figures moving on lines. The child runs to velocities of 0.1 to several seconds. A sound and congratulations message is presented if the child counts the correct number of complete figures. Another sound accompanies erroneous answers. The adequate solution of the task showed age dependence. In this way, normal children were capable to count correctly until 0.1 sec. velocities the simplest figures. But subjects up to 12 years old need 0.5 sec. to answer correctly the more complex exercises. For dyslexic children it was very difficult to obtain correct answers, even tough the programs were runned very slowly. This results indicate visual spatial attention deficits for disabled readers.
478.11
A HIERARCHICAL MODEL FOR 3D OBJECT RECOGNITION BASED ON 2D VISUAL REPRESENTATION.
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A central question in visual recognition is whether internal representations of 3D objects are themselves 3D and object-centered or 2D and viewer-centered. Recent evidence suggests that in addition to some classes of 3D objects that are recognized by the 2D properties of the stimulus image, specifically, (1) the ability to parse different views of a 3D object in 3D space, and (2) the influence of 3D transformations (affine and non-affine) on recognition is correlated with the induced image-plane distortion and not with the way of the 3D transformation itself (Edelman & Bialek, MIT AI Memo 1295).

These findings are consistent with a representation scheme based on multiple 2D views along with some means for view interpolation (e.g., Poggio & Edelman, Nature, 330). A computational network model of visual processing for view interpolation has been developed (Intrator & Gold, in press) using a biologically motivated learning and self-organization scheme (Bienenstock et al., Neural Networks). The model not only simulates the psychophysical data, but can also produce a sophisticated statistical analysis for dimensionality reduction (Intrator, Nature, 479.14) using a biologically motivated learning and self-organization scheme (Bienenstock et al., Neural Networks). The model not only simulates the psychophysical data, but can also produce a sophisticated statistical analysis for dimensionality reduction (Intrator, Nature, 479.14).

We have trained 5 cats in orientation discrimination with illusory contours (IC) in which the contour was induced by two sets of circle halves of which the endpoints were either separated by a gap or shifted in phase. Five cats were trained in texture (TEXT) segregation (square of line segments of a 3D object to mask the stimulus). Five cats were trained in TEXT-segregation (square of line segments of different orientation). In three IC- and three TEXT-cats we made bilateral ablations of areas 17 and 18 (type-1 lesion). Performance was close to normal in both cases, with little or no recovery despite intensive retraining for 0.5 to 1 year. In 4 cats (2 IC- and 2 TEXT-cats), we made combined bilateral lesions of areas 17 and 18, which receive direct projections from 17 and/or 18 (type-2 lesion). The initial effect (first two months) was as devastating as after type-1 lesions, for both IC- and TEXT-cats. However, retraining allowed 3 of the 4 cats to re-attain close-to-normal performance. Our data suggest that the areas destroyed by type-1 and type-2 lesions influence in the normacortical processing of illusory contours and textures, and that intactness of areas 17 and 18 is critical in the recovery observed after type-2 lesions.

478.13
We recently described a patient (DF) with visual form agnosia who showed strikingly accurate guidance of hand and finger movements directed at objects whose size, shape, and orientation she failed to perceive (Goodale et al., Nature 1991). MRI revealed damage in areas 18 and 19, with considerable sparing of the primary visual cortex. The dissociation in visual performance suggests that visual mechanisms underlying "conscious" perceptual judgements normally operate separately from those underlying the "automatic" visuomotor guidance of skilled actions of the hand and limb. We have since shown that DF is unable to use visual patterns to guide her hand movements although the lesion is sensitive to the orientation of the elements that comprise the pattern. Thus, when presented with a slotted surface in which the slot was cut in the shape of the letter T, DF was able to guide a T-shaped form into the slot on only half the trials. Interestingly, her visually-driven errors were almost always perpendicular to the correct orientation. In other words, the mechanisms controlling her visuomotor behaviour appeared unable to "discriminate" the stem from the top of the T, even though the hand-held form was rotated to the correct orientation of one element of the T or the other. This result combined with our earlier observations suggests that in normal brains there may be a contribution to visuomotor guidance from the systems underlying pattern recognition although more basic information about object dimensions and orientation can probably be processed independently from these pattern recognition systems. Supported by ERC grant MA-T-269 to MAG and an ERC postdoctoral fellowship to J.S.P.

478.14
Four monkeys were trained on a serial-order task (D'Amato and Colombo, Elberling and Kadar, Neurosci. Lett., 15, 131-139) to press five simultaneously presented visual stimuli in one specific order, symbolized as A B C D E. Two monkeys then received bilateral lesions of inferior temporal cortex and two received bilateral lesions of dorsal frontal cortex. The frontal lesions had no effect on performance of the serial-order task. In contrast, inferior temporal lesions severely disrupted performance.

After relearning the task postoperatively, the monkeys were given a test of visual imagery in which two of the five stimuli were presented at a time and the subject was required to respond to them in the order in which they appeared in the sequence. A B C E or B C E A. A dorsal inferior temporal and dorsolateral frontal lesions impaired performance on this test. The significance of these findings for understanding the neural mechanisms of visual imagery and their relation to the response properties of inferior temporal neurons is discussed.

478.16
WAVELET METHOD AS THE NEW APPROACH TO ANALYSIS OF EVOKED POTENTIALS IN THE ELECTROENCEPHALOGRAM. A. W. Prewyzszewski, O. J. Gruber. Dept. of Physiology, Freie Universität Berlin, Amimalt 22, 1 Berlin 33, Germany.
A new method in analyzing stimulus-category-related visual evoked potentials (EPs) is demonstrated.
(a) EPs related to face, hand, and object perception were convoluted with the second derivative of the Gauss function (so-called wavelet function) to obtain wavelet transformation. The width of the wavelet function was varied from 44 to 264ms, resulting in 3D-pictures.
(b) The same procedure was applied for category-specific difference EPs. (c) To obtain statistical evaluation criteria the individual EPs were randomly shuffled across stimulus categories, and from these "random groups" average "random EPs" were computed. From these "average random EPs" difference curves were computed and treated with the wavelet method as described above.
(d) This "statistical error" basis was compared with wavelet transformation of category difference EPs and the range where this difference exceeded ± 3 s.e. was determined (figure).
(c) This range was mapped on a 2D-diagram ("contour plot"), (seen figure in Seidler et al. abstract this meeting). (Supported by DFG grant Gr. 161).

The influence of gender and face appearance on visual evoked potentials (VEPs) was studied. In 10 subjects (8 male, 2 female, range: 23–31 years) VEPs were recorded through the electrodes F3, F4, T5, T6, Cz, Oz, with reference to linked mastoids (intern. 10/20-system). 160 different slides were projected for 2.5 s each with slowly varying, Black/white-plasticine "attractive" male and female faces, which had been previously rated by other independent subjects, served as stimuli. The face-responsive component appearing in the VEP (Botzel & Grüsser, 1991) was confirmed, consisting of a pronounced positive peak at about 200 ms with a consecutive very rapid negativity. Significant differences were found between the stimulus category "attractive females" and the other 3 categories. The presentation of attractive females and male faces led to larger positive amplitudes beyond 150 ms latency, which were significant at the right frontal electrode F4 and Cz (Fig. 1). This result was more pronounced in VEPs of male subjects compared to female subjects (Fig. 2).

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479.17

479.18


Botzel and Gründer described components in EEG-evoked potentials (EP) related to the presentation of low-photos of face stimuli. The largest amplitude of the EP related to faces and the most distinct category-related differences were found at electrodes F3, F4 and Cz (reference linked mastoids) as EEG-response of 3 prominent peaks (N135-P200-N290) were found. The presentation of photos of 11 different paradigms to 133 right-handed subjects (50% female, 50% male). In 4 paradigms ("class 1") we compared face- to non-face-stimuli (e.g. body-parts, tools), in 3 paradigms ("class 2"), to other face-stimuli (e.g. different views of the head). The category-related responses for body- and face-parts were similar to those evoked by faces and revealed a large positive peak at about 200-210 ms. Beyond 210 ms the EPs deviated significantly from "face-EPs". The EPs related to flowers and tools were very similar and had less prominent and later peaks (Fig. 1). Comparing the results between the two classes of experiments, significant differences in "face-EPs" occurred only between 340-440 ms. In the earlier face-specific-components, no difference could be detected (Fig. 2). Gender differences appeared for face-stimuli beyond 340 ms. (DFG-grants Gr616 & Se520)

Cochlea: nerve responses, transmitters and second messengers

480.1

Dil labeling of the auditory nerve in the chinchilla and mongolian gerbil. P. F. Schäfke1,2, A. Al-Ahmad3, S. C. Chamberlin1, N. B. Slepecky 1, R. L. Smith 1,2, and B. T. Verrelli1. Institute for Sensory Research, Department of Electrical and Computer Engineering, and Department of Bioengineering, Syracuse University, Syracuse, NY 13244

The auditory nerves of chinchillas and gerbils were labeled with minute crystals of Dil. In chinchillas, the dye was placed either in the surface of the auditory nerve in the internal meatus or in Rosenthal's canal at various sites along the cochlear spiral. In gerbil, dye was placed only at sites in Rosenthal's canal. Diffusion of the dye occurred in darkness at room temperature for 4-11 months while the tissue was stored in 4% paraformaldehyde. After 0.1 M phosphate buffer at pH = 7.2. Tissue was then dissected according to fluorescence or resolution.

When Dil was placed on the auditory nerve, the neurons of radial fibers to inner hair cells were continuously labeled along a region in the apical half of the basal turn; however, no labeled spiral fibers were observed in the organ of Corti. This suggests that the axons of type 1 II ganglion cells are segregated from type I fibers in the internal meatus. In both species, puncta labeling in the spiral ganglion resulted in a discrete dense band of labeled fibers in the modiolus; a mottled region in the peripheral internal meatus; and a discrete dense band as the nerve trunk entered the cochlear nucleus. The mottled region suggests that a zone of tonotopic scrambling may exist at the base of the modiolus. We examined carefully the labeling of stained fibers in the gerbil cochlear nucleus. No Dil was observed surrounding the characteristic micronuclei lesions confirming that these structures are synaptic and not part of the auditory nerve fibers. The ultimate objective of continuing studies is a detailed map of the tonotopic organization of the auditory nerve in the modiolus, internal meatus, and cochlear nucleus in both species.

Supported by NIH grant PO1/DCO80 NSF grant BNS8920418, and the Department of Bioengineering.

480.2

Mechanical and neural contributions to prolonged response latencies in immature kittens. E.J. Walsh and J. McGinty, Boys Town National Research Hospital, Omaha, NE 68131

It is well known that neural responses to auditory stimulation are significantly prolonged in neonatal kittens relative to those observed in adults (Walsh et al., 1986). The source of the prolongation is unknown and both cochlear and neural factors may contribute to the immaturity of the cochlear microphonics (CM) measured at 4 kHz. Latency of the cochlear microphonics (CM) was measured to estimate cochlear delay associated with responses to acoustic stimulation, and auditory brainstem responses (ABR) which were measured simultaneously to determine overall response latency. The difference between the onset of CM and ABR Wave I latency was taken as an inferential estimate of synaptic and other neural delays. ABR and CM data were collected in six kittens. The early components related to body- and other neural and synaptic delay was measured. These procedures were used to record CM and ABR so that measurements could be made from individuals longitudinally.

CM and ABR Wave I latencies declined along roughly exponential trajectories and CM acquired adultlike latencies relatively early compared to Wave I (i.e., CM latencies were adultlike by the end of the second postnatal week). At 4 kHz, CM latency accounted for approximately 70% of the overall response latency on the 7th postnatal day, 67% on day 11, and 50% on the 15th day. Significant differences were made for responses to 1 kHz, although CM accounted for approximately 60% of the total delay at both 11 and 15 postnatal days. Thus, preliminary results indicate that mechanical immaturities originating in the cochlea are the primary determinants of Wave I latency prolongation and that neural immaturities associated with synaptic delay and myelination account for the remainder.

(Supported by NIDCD grant DC01007).

480.3

Functional characteristics of spiral ganglion neurons after hair cell regeneration. R.J. Salvi, S.S. Sauden*, and E. Hashkin*. Hearing Research Lab, SUNY University at Buffalo, Buffalo, NY 14214

Recent studies have shown that sensory hair cells in the avian ear can regenerate after being destroyed by ototoxic drugs or acoustic trauma. One of the important questions that need to be answered is whether regeneration can restore the normal function of the reinnervated newly formed hair cells. At present, some evidence is available that functional hair cells were present in chickens using kanamyicin (KOM) (400 mg/kg/d x 10 days). Immediately after KOM treatment, the compound action potential (AP) threshold was significantly elevated and the amplitude greatly depressed. The only neurons that responded to sound immediately after KOM treatment were those to low-frequency tones. When KOM-treated neurons were exposed to the characteristic frequency (CF), thresholds of the units were extremely high and their tuning curves were broader than normal. In addition, the spontaneous discharge rates of neurons that were responsive to sound immediately after KOM treatment were not significantly increased. Over the next few weeks, the AP threshold and amplitude recovered rapidly and neurons with a broader range of CFs were encountered. Similarly, the neural thresholds improved, the tuning curves became more independent of fiber "characteristic frequency (CF)", and the spontaneous discharge rates increased with recovery time; however, these measures had not fully recovered by 40 post-treatment. (Supported by NSF BNS9007822).

480.4

Characteristics of the response recovery following two-tone suppression in auditory-nerve fibers. K.G. Hill1 and C.D. Geisler2, 1Research School of Biological Sciences, Australian National University, Canberra City, Australia; 2Depts of Neuropsychology and Electrical and Computer Engineering, University of Wisconsin, Madison, Wis. 53706.

During a period of tens of milliseconds following termination of an excitatory, conditioning tone, the neural rate response in an auditory nerve fiber to a second excitatory test tone is reduced, relative to the response to the test tone preserved in isolation. Normal response magnitude to the test tone characteristically recovers as the delay between termination of the test tone and the test tone is increased (Harris and Dallos, Neurophys. 82, 1083, 1979). This effect, is also manifest as post-excitatory depression of spontaneous rate.

Immediately following termination of a suppressor tone imbedded in a continuous, excitatory tone the neural rate response in an auditory nerve fiber to a second excitatory test tone is reduced, relative to the response to the test tone preserved in isolation. Normal response magnitude to the test tone characteristically recovers as the delay between termination of the test tone and the test tone is increased (Harris and Dallos, Neurophys. 82, 1083, 1979). This effect, is also manifest as post-excitatory depression of spontaneous rate.

Immediately following termination of a suppressor tone imbedded in a continuous, excitatory tone the neural rate response in an auditory nerve fiber to a second excitatory test tone is reduced, relative to the response to the test tone preserved in isolation. Normal response magnitude to the test tone characteristically recovers as the delay between termination of the test tone and the test tone is increased (Harris and Dallos, Neurophys. 82, 1083, 1979). This effect, is also manifest as post-excitatory depression of spontaneous rate. In a two-tone paradigm, if the suppressor tone is set to a level that elicits a neural rate equal to the steady-state, CF-driven rate, or, if the suppressor tone and the CF tone at corresponding levels are presented in sequence with the suppressor tone loading as conditioner, then a similar delayed recovery of the CF-driven rate is observed. This suggests that recovery of response to the CF tone following presentation of the suppressor, either in the two-tone paradigm or as a prior conditioning tone, shares a common mechanism.

Supported by DC01016 (NIH)

Peripheral hearing was assessed in newborn beaglet puppies using brainstem auditory evoked potentials (BAEP) and other derived responses; recordings for presentation of auditory stimuli. Implants were also made in two Dalmatians to carry the deafness gene(s). Click stimuli (100 µs, 11.4, 135 dB SPL; repetition [R], condensation [C] and alternating [A] polarity) were used to elicit basilar membrane evoked potentials (BAEP) and other derived responses; recordings (n=1000 X 2, 10 ms, 150-3K Hz, vertex to tympanic electrode) were made daily until after the opening of the ear canal.

In the beagle puppies, BAEP peaks were present on days 44 after birth in response to R and C stimuli, along with the N1, the 2nd nerve action potential, while responses to A stimuli were not present until 1-2 days later. A similar pattern was observed in one Dalmatian, although possibly delayed. The second Dalmatian showed a CM but no BAEP or N1 up through the age of 9 days when it died; it is assumed that this dog would have been deaf in the implanted ear.

The observed sequence of development was earlier than previously reported for the dog (Acta Oto-laryngol. 76:1-13, 1973). (Supported by La-SVM 1272, NIH DC00128, and the Dalmatian Club of America.)


A microcomputer-based system has been developed for fast, digital synthesis of complex sound stimuli and recording of single-unit neurophysiological responses. The software is based on the Baylis (1977) auditory scene analyzer and provides an interactive graphic analysis of response properties. For synthesis and spike recording, the system uses an Apple Macintosh II, a 12-W I/O board, and a custom interface and signal conditioning electronics. The software includes a library of actual natural sound files, which are selected by a menu. The software also allows the designer to add additional data files.

For presentation of auditory stimuli, experiments were conducted on guinea pig cochlear preparations, with both transcranial and intracranial pressure stimuli. The stimulus was a bank of white noise pulses, with a 500 ms duty cycle. The stimulus was presented to the left ear with a low-pass filter set at 20 kHz, and the output from an amplifier was routed to a 16-bit analog-to-digital converter and sent to the microcomputer. The software allows the user to set the stimulation parameters, including tone onset and offset times, and the stimulus intensity. The microcomputer then generates the stimulus, which is then routed to the amplifier and presented to the animal.

The system is capable of generating complex sound stimuli, including those used in previous studies. In one study, the stimulus consisted of a bank of white noise pulses, with a 500 ms duty cycle. The stimulus was presented to the left ear with a low-pass filter set at 20 kHz, and the output from an amplifier was routed to a 16-bit analog-to-digital converter and sent to the microcomputer. The software allows the user to set the stimulation parameters, including tone onset and offset times, and the stimulus intensity. The microcomputer then generates the stimulus, which is then routed to the amplifier and presented to the animal.
Molecular Cloning of cDNAs for G Proteins from Cochlear Tissues. M. Tachiara*, E. Wilcox*, N. Yokota*, M. Schneider*, D. Drewartz, and J. M. Hall. Lab. of Molecular Biology, National Institute on Deafness and Other Communication Disorders, Bethesda, MD 20892

G proteins play crucial roles in sensory organs such as retina and olfactory epithelium. Little is known, however, about G proteins in the hearing organ. The purpose of this study was to obtain the primary structure of G proteins in the cochlea by molecular cloning techniques.

Total RNA was extracted from the whole cochleas of the mouse and from the organ of Corti or stria vasculosa/spiral ligament of the guinea pig. RNA was reverse transcribed and cDNAs with nucleotide sequences resembling those coding for G proteins were selectively amplified by polymerase chain reaction (PCR), using degenerate primers. Among the PCR products, two bands of approximately 300 and 350 base pairs. Individual PCR products were cloned into pGEM-7Zf+(+) plasmid vector. Using these G protein-like cDNAs as probes, we are now trying to determine full-length sequences of G protein cDNAs from a cDNA library, which we are making from the mouse cochlea.

Fibronectin Staining of the Basilar Membrane of Young and Aged Rats. E. M. Keithley, I. Kitabayashi, and N. K. Woolf. Division of Otolaryngology, University of California, San Diego, La Jolla, CA 92037.

Mechanical presbycusis was hypothesized by Schuknich (Arch Otolaryngol 80:36964) to account for age-related hearing loss in excess of that attributable to the loss of hair cells and neurons in the human cochlea. Aged animals also show hearing losses that seem large relative to the loss of sensory-neural elements. It has been shown that repair is based, at least in part, on production of new hair cells. The mechanism by which avian hair cells regenerate is not known, it has been proposed that hair cell regeneration may occur by one of two different mechanisms in chicks: 1. Supporting cells may transdifferentiate into cells with this "immature hair cell-like" combination of morphological features is that supporting cells may transdifferentiate into new hair cells. Four Gi-like cDNAs were obtained from the guinea pig stria vasculosa/spiral ligament. Three of these had moderate (74-81%) nucleotide similarity to the known G1 cDNAs from other tissues. One of them showed high (97%) similarity to the G1 cDNA from the macropoage cell line. Using these G protein-like cDNAs as probes, we are now trying to determine full-length sequences of G protein cDNAs from a cDNA library, which we are making from the mouse cochlea.

Dual Mechanisms for Production of New Hair Cells in Regenerating Avian Cochlea. Y. Raphael* and J. M. Miller. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

Acoustic overstimulation of chick auditory epithelium results in hair cell loss and subsequent replacement by new hair cells (Corwin and Cotanche, 1988; Ryals and Rubel, 1988). Although the complete mechanism by which avian hair cells regenerate is not known, it has been shown that repair is based, at least in part, on production of new hair cells. The mechanism by which avian hair cells regenerate is not known, it has been proposed that hair cell regeneration may occur by one of two different mechanisms in chicks: 1. Supporting cells may transdifferentiate into new hair cells. Four Gi-like cDNAs were obtained from the guinea pig stria vasculosa/spiral ligament. Three of these had moderate (74-81%) nucleotide similarity to the known G1 cDNAs from other tissues. One of them showed high (97%) similarity to the G1 cDNA from the macropoage cell line. Using these G protein-like cDNAs as probes, we are now trying to determine full-length sequences of G protein cDNAs from a cDNA library, which we are making from the mouse cochlea.
480.17
FUROSEMIDEOTOXICITY IS ENHANCED IN ALBUMIN-DEFICIENT RATS. L.P. Rybak, C. Whitworth, V. Scott, A. Weberg, Dept. of Surgery, SIU School of Med., Springfield, IL 62794

The analbuminemic rat was developed from Sprague-Dawley rats (Nagase et al., Science 205:590, 1979). The Nagase analbuminemic rat has only barely detectable amounts of albumin in its serum. These mutants have no grossly discernible signs of pathology, and the total serum protein concentration is similar to that of normal Sprague-Dawley rats because of an increased globulin fraction. Furosemide is a loop diuretic which is highly protein-bound. Ototoxicity is one of its potential side effects.

The purpose of the present studies was to investigate the effect of furosemide on the endocochlear potential (EP) of the Sprague-Dawley and NAR rats. Young adults of either type were anesthetized with Rompun (1 mg/kg). Tracheotomy was performed and the EP was measured with a microelectrode inserted through the round window membrane. After a stable EP was recorded, furosemide 35 mg/kg was injected through a cannula in the jugular vein. Sprague-Dawley rats were found to have very small reduction of the EP (9.46 ± 2.9 mv). In sharp contrast, the NAR's exhibited an extremely large reduction of the EP (88.0 ± 10.0 mv) (p<0.001). These findings support the hypothesis that the access of furosemide to its site of ototoxic action in the cochlea depends on the quantity of unbound furosemide in the serum. (This work supported by NIH-NIDCD Grant #DC-00321 and NIGMS #GM-40858).

480.2
PARTIAL PURIFICATION AND CHARACTERIZATION OF AN L-ARGININE RECEPTOR/CHANNEL FROM CATFISH TASTE EPITHELIUM. J.G. Brand1,2, J.H. Teeter1, and D.L. Ballard3, Monell Chemical Senses Center, Univ. of Penn., and Veterans Affairs Administration, Phila, PA 19104

Behavioral, neurophysiological and biochemical studies suggest that there are independent high affinity receptor sites for the stimulus L-Alanine (L-ALA) and L-Arginine (L-ARG) in the catfish taste system. Biochemical studies have characterized binding of L-ALA and L-ARG to a sedimentable membrane fraction (P2) from taste epithelium. We have initiated a search for the various genes involved in the cyclic nucleotide and phosphoinositide signal transduction pathways in our cDNA library.

We describe the construction and the characterization of the rat taste epithelial library as well as the identification of various signal transducing genes found in it. Also we report the cloning and characterization of a unique potassium channel gene that has high homology to a delayed rectifier potassium channel.

481.1

The mammalian taste receptor cells utilize diverse signal transducing mechanisms for different taste modalities. These are thought to range from direct interactions with apical membrane ion channels by certain ionic tastants to the generation of intracellular second messenger molecules by other types of tastants. Data from our laboratory as well as from others suggest a role for second messenger systems in taste signal transduction. However, the scarcity of rat taste receptor cells has limited biochemical investigations into the mechanisms of taste transduction. In order to circumvent this difficulty we have recently made a cDNA library from rat tongue epithelial tissue highly enriched in taste receptor cells, and we have initiated a search for the various genes involved in the cyclic nucleotide and phosphoinositide signal transduction pathways in our cDNA library.

We describe the construction and the characterization of the rat taste epithelial library as well as the identification of various signal transducing genes found in it. Also we report the cloning and characterization of a unique potassium channel gene that has high homology to a delayed rectifier potassium channel.

481.3
ANTAGONISM OF THE GERBIL'S SUCROSE TASTE RESPONSE BY 6-CHLORO-N-ACETYLD-TRYPTOPHAN. William Jakunovich, Jr. Dept. of Biological Sciences, Lehman College and the Graduate School, CUNY, Bronx, NY 10468

We have discovered a fourth sucrose taste antagonist, 6-Chloro-N-Acetyl-D-Tryptophan (6-CNAT), an amino acid rather than a sugar derivative, which has been partially characterized as follows:

a) In solution, by itself, it does not stimulate the gerbil's chorda tympani nerve.
b) The taste response produced by sodium chloride was unaffected by 6-CNAT.
c) Sucrose responses were inhibited when mixed with 6-CNAT.

Supported by NIH NINCDS grant #DC00454.

481.4
DYNAMIC FILTER PROPERTIES OF CHEMORECEPTOR CELLS IN SIMULATED ODOR PLUMES. J. Atema, R. Voigt, and G. Gomez*, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA, 02543

Intensity fluctuations in turbulent odor plumes are typically chaotic in time and space dimensions. These stimulus intensity patterns are filtered by a fluid boundary layer before they reach the receptor surfaces. Microvoltammetry allows us to measure a more precise temporal correlation between stimulus arrival and cell response than previously possible. A carbon-tip microelectrode approximately the size of a lobster olfactory sensillum (30 um diameter) was placed into the tuft of sensilla in a recording chamber allowing simultaneous measurements of the chemical signal and the neural response to the stimulus. We used both well-defined pulses and with semi-natural odor plume patterns. The instantaneous firing rate of spikes in relation to the instantaneous stimulus intensity profile allowed us to determine the response properties of receptor cells in a similar manner. The signal characteristic of odor plumes into a temporal neural signal pattern (transfer function). The data confirm results from earlier studies on adaptation and disadaptation properties of receptor cells and predictive computer models based on information contained in odor plumes (Moore & Atema, 1988 Biol. Bull.). The rate of stimulus onset and its relation to the immediately preceding stimulus history appear to be critical features for these chemoreceptor filters.

Supported by NSF (BNS 8812952) to JA.
481.5

IN SITU RECORDING FROM HAMSTER FUNGIFORM TASTE CELLS: RESPONSE TO SOUR STIMULI. J.A. Gilbertson, P. Ayvaz, S.C. Kimm assumed and R.D. Roper. Dept. of Anest and Neurobiol, Colum St Univ, S. Collins CO 80223, and Rocky Mt. Taste and Smell Center, Univ Colo Health Science Center, Denver, CO 80262.

Current transient reflecting taste cell action potentials were recorded in response to sour stimuli using a non-invasive technique (Ayvaz and Lindenbaum, Biophysical Abstracts 59:594, 1991). A recording pipette of 100 μm tip diameter which could be internally perfused with 10% (v/v) stimulant solution was used to perfuse the taste cells. Stimulus solutions contained 30 mM NMDG, Cl, 5 mM HEPES, pH 7.0 for 30 mM NMDG-Cl and various combinations of either citric acid (CA) or HCl. Action currents were recorded up to 20 pA in amplitude and their frequency varied in a dose-dependent manner with pH. Shapes of action currents were generally biphasic, but monophasic currents were also recorded. In a single taste bud cell, CA always gave a stronger response than HCl at an equivalent pH, but NMDG-citrate buffered at pH 7.0 gave no response. Taste buds previously adapted to NaCl (100 mM) still showed responses to CA indicating that different afferents are involved in sour and sweet responses.

Unlike the mullipapillate (Kimmann and Roper, J. Gen. Physiol. 91:351, 1988), the citric acid response was not affected by HCl or TEA. The response to CA was also unaffected by vanadate, 9-AC, cadmium, DIDS, nor was it affected by exposing the apical membrane to NH4Cl to modify internal pH. Amiloride (30 μM), however, completely and reversibly suppressed the action currents elicited by CA. An amiloride-sensitive steady state current could also be measured in the presence of CA. These data suggest that sour (acidic) stimulation involves an amiloride-sensitive process and that in mammalian fungiform papilla the transduction mechanism for sour taste does not involve a blockage of apical K-channels. Supported by NIH grants DC00244, DC00766(SCK), DC00374(SDR), and AG06557(SDR).

481.6


In vertebrate taste buds, intracellular dye injections with Lucifer Yellow have revealed coupling between receptor cells (Yang & Roper, 1987). To study functional intercellular coupling in taste buds, we have developed a new experimental approach consisting of applying patch pipettes onto taste cells in thin (100-200 μm) slices of lingual epithelium from Neocerus maculatus. In this preparation, internuclear responses among cells within the taste bud are preserved. The whole-cell voltage-clamp configuration of the patch recording technique readily allows one to measure the membrane capacitance of taste cells. Membrane capacitance gives an estimate of the cell membrane available to be charged during a voltage step command, including any additional membrane contributed by electrically-coupled cells. We tested whether capacitance measurements of functional electrical coupling could be performed by perfusing the slice preparation with 1-octanol, a blocker of gap junctions. If taste cells are electrically-coupled via gap junctions, we should initially record high values of cell capacitance which would be reduced by 1-octanol. Indeed, in some experiments, cell capacitance was initially high and decreased when 500 μM 1-octanol was added to the bath. Lucifer Yellow in the patch pipette confirmed that these cells were coupled to adjacent cells. In other experiments, 1-octanol had no effect and these cells were not dye-coupled. These data indicate that functional electrical coupling between taste cells can be monitored by measuring membrane capacitance during patch clamp recordings. This now provides a means to test whether electrical coupling in the taste bud is affected by chemosensory stimulation or by neuromodulation.

481.7


Recent studies have shown that unilateral neonatal naris closure, assessed in adult rodents, produces a decrease in the expression of the dopamine phenotype in periglomerular neurons of the ipsilateral olfactory bulb. To distinguish afferent regulation of development from maintenance of phenotype, the current studies investigated the effects of unilateral naris closure produced in adult (4- to 6-month old) C57BL/6J mice. The changes in the dopamine phenotype, i.e., activity, immunoreactivity and mRNA for tyrosine hydroxylase (TH), the first enzyme in catecholamine biosynthesis, were measured 6-8 months post-closure. TH activity ipsilateral to the closure was reduced to 25% of that in the contralateral side and was 20% of that in sham operated animals. TH immunoreactivity (evaluated with a specific TH-antiserum) and mRNA (demonstrated by in situ hybridization (ISH)) exhibited a similar down-regulation. In contrast, immunoreactivity and mRNA (by ISH) for the GABA synthesizing enzyme, glutamic acid decarboxylase, were unchanged both ipsilaterally and contralaterally to the closure. These data suggest that maintenance as well as development of the dopamine phenotype are dependent on odor stimulation. Supported by AG09686.

481.8


We have examined the immunohistochemical reactivity of monoclonal antibodies directed against a 70 kDa heat shock protein (HSP70) and ubiquitin in the OE of control and unilaterally bulbectomized (OB-X) rats. These proteins are expressed rapidly and transiently in response to a variety of stresses. OE from Bouin's-perfused unoperated rats showed widely scattered HSP70-positive olfactory sensory neurons in the apical half of the OE. This localization and their absence from OB-X OE at 10 d postop. indicates they are very mature neurons. Following OB-X resection, reactivity to ubiquitin and HSP70 appears in the supporting cells of 2 and 6 hrs postop, respectively. These initial responses were bilateral and also occurred in sham operated controls. However, by 24 hr supporting cell reactivity occurred on the OB-X side only. By 48 hr postop this supporting cell reactivity had disappeared. The results support other data showing rapid, transient stress protein responses in other systems (Brown, Neurosci. Res., 27:247, 1990). Supported by NIH Grant #DC 00347.

481.9


Use of salamanders with large taste buds of a simpler structure has promoted cellular studies of taste receptor cells. However, little is known about afferent innervation to the cells. In a whole mount preparation of the anterior of the lingual epithelium, the overall innervation pattern of the glossopharyngeal nerve (IX) was clearly shown by the fluorescent carbocyanine dye, diocetyl-tetramethylindo-carbocyanine perchlorate (DiI) applied to the IX nerve stump. Beneath the epithelium, the labeled nerve fibers expanded horizontally to form a mesh-like structure, from which fascicles of the fibers extended outward toward the epithelium to innervate taste buds. Numerous taste buds (10/square mm at the tip) were labeled possibly transneuronally. In sectioned preparations, a single or several labeled cells with apical elongation toward a taste pore, showing a typical taste cell, was seen. In-between the distributed taste buds, fine fibers apparently formed free nerve endings within the epithelium.
485.1 RESPONSIBILITIES OF SUBSTANTIA NIGRA CACII TO PALLIDAL STIMULATION AND DOPAMINE RECEPTOR BLOCKADE J.R. Hollerman and A.A. Grace, Department of Pharmacology, University of Pennsylvania, Philadelphia, Pa. 19104.

Neurochemistry

3 Horizontal striatum receives thousands of excitatory synaptic inputs from different afferent fibers, which somehow act together to determine the timing and duration of the episodes of firing that are characteristic of these cells. Computer simulations of synaptic inputs, however, do not yet include all known synaptic inputs to the horizontal striatum. Consequently, the degree of cooperativity that can be observed may be a "rebound" interaction. Administration of HAL (to the pituitary area) is known to inhibit the release of dopamine, while administration of L-DOPA (to the substantia nigra) is known to increase the release of dopamine. Thus, simultaneous administration of HAL and L-DOPA may have a synergistic effect on the release of dopamine. This would be consistent with the finding that the release of dopamine is increased in striatum following administration of L-DOPA. However, the exact mechanism by which this effect is produced is not yet known. Further studies are needed to clarify this point.

485.2 NEUROTRANSMITTER ACTIVITY RELATED TO MOVEMENT PREPARATION IN A GO/NO-GO TASK IN THE CAT. J.W. Aldridge, J.F. Thompson, E.A. Walters, J.M. Groth and G. Gilman, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

The functional role of single units in the putamen (PUT) and caudate nucleus (CN) in relation to the preparation of visually cued reaching movements was studied. Two cats were trained in a GO/NO-GO visual discrimination task. The animals sat on a force platform that had sensors for each limb, head movements, and contact with the floor. The food delivery apparatus and target presentations were computer controlled. Visual cues were presented at a control period in which all limbs had to remain in contact with the force platform. Correct actions were: 1) GO cue - touch display with right forepaw; 2) NO-GO cue - no display with force platform. Simple units were recorded with microelectrodes inserted through a recording chamber. Changes in discharge rate were identified by a statistical comparison of epochs in the control period and cue-to-movement period. Significant rate changes in the GO task were detected in 31.2% of PUT (30/94) and 24.2% of CN (56/205) units. Lateral CN regions had more responsive units (52.1% vs 17.4%) than medial regions. Discharge changes prior to movement were usually (60.8% PUT, 56.1% CN) more tightly coupled to the stimulus than the up-cuing movement. Units with vigorous GO cue-related activity were usually (72.2% PUT, 54.5% CN) activated in the NO-GO task. Units with a weak link to the GO cue were rarely activated in the NO-GO task and units responsive to the NO-GO cue alone were uncommon. These findings suggest that, in the behavioral setting, the cat's motor cortex is strongly related to preparation for executing or withholding movement. Furthermore, unit discharge related to the sensory cues is strongly linked to the context of movement requirements in the task. Support: NIH grant NS19613 and United Cerebral Palsy Foundation.
482.7 THE RELATION OF PALLIDAL ARM NEURON DISCHARGE TO THE AMPLITUDE AND VELOCITY OF MOVEMENT. R.S. Turner and M.C. Anderson. Department of Physiology and Biobehavioral Psychology, The University of Iowa, Iowa City, Iowa 52242.

Pallidal arm neuron discharge is often related to movement amplitude or velocity (Georgopoulos et al., 1983). We addressed two questions: (1) is pallidal arm neuron discharge related to movement amplitude or velocity? (2) do movement amplitude effects interact with other characteristics of movement related changes in discharge?

Pallidal arm neurons were recorded from the external and internal segments (GPx and GPi) of two monkeys while they performed a two dimension reaching task. This required hand movements across a surface to targets in 8 directions at 5 distances from a central starting point. Arm neurons were identified by: (1) task related changes in firing; and (2) responses to passive manipulation of the arm.

A high proportion of the neurons had task related changes in firing that were scaled with movement amplitude. Fewer were related to movement velocity. Of the 30 GPx and 12 GPi arm neurons studied during movements to three target distances, 67% had a linear relation between the average firing rate during the movement time and movement amplitude. All neurons in which movement trains were tested (8 neurons) had a significant movement amplitude effect. The amplitude effects were independent of the magnitude, sign, and directionality of a neuron's task related discharge. Firing rate was better related to movement amplitude than peak velocity in 71% of the cases studied. Partial correlation coefficients confirmed this.

Thus, pallidal activity is preferentially and independently related to movement amplitude. Supported by NS15017, RR00016 and H133B0081.

482.8 CHRONIC MULTI-CHANNEL SINGLE-UNIT RECORDINGS FROM 6-OHDA-TREATED RATS DURING D-LOPUS-INDUCED CIRCLES. S.C. Sawyer, C.D. Myers and D.J. Woodzell. Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75235

Damage to the dopaminergic nigrostriatal projection in rats following intracerebral injection of 6-hydroxydopamine (6-OHDA) constitutes a model of Parkinson's disease. We have previously reported altered rates and patterns of neuronal activity in the neostriatum of rats following unilateral 6-OHDA lesions. The present study was conducted to determine the extent to which dopaminergic nigrostriatal unit activity was monitored following administration of D-LOP in unilateral dopamine-depleted rats. Male Sprague-Dawley rats received an injection of 6-OHDA (8 μg in 4 μl) into the left medial forebrain bundle. Two weeks later the animals were implanted in the basal ganglia, with at least one arm position in the left neostriatum. Damage to the dopaminergic nigrostriatal projection was assessed by behavioral testing and contemporary microdialysis. Parkinsonian-like circling behavior was observed in all animals treated with D-LOP (50 mg/kg, i.p.). Microdialysis localization was determined from X-rays and histological reconstruction. Spike activity of up to 8 neurons was recorded concurrently for weeks following 6-OHDA administration. Preliminary studies indicate that neither scopoline 0.3 mg/kg, i.p.; D1 antagonist) nor sulpiride (30 mg/kg, i.p.; D2 antagonist) blocked or reduced the D-LOP-induced alterations of neostriatal spike activity. Administered together, the antagonists blocked both rotation and alternations in unit activity. Further studies are underway to confirm the basis of these D-LOP-induced alterations.

482.9 ACTIVITY OF DOPAMINE NEURONS IN MONKEYS LEARNING AND PERFORMING COGNITIVE TASKS. W. Schultz*, T. Ljungberg* and P. Apicella* (SPON: European Neuroscience Association). Institute of Physiology, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

Cognitive deficits are major symptoms of Parkinson's disease. Previously we showed that dopamine (DA) neurons in behaving monkey respond to attention-generating and motivating stimuli. We now tested DA neurons in cognitive tasks. In spatial 8-arm radial and 2-bottle operant, two horizontal arm arrays were illuminated for 1 s as instruction. A central arm illuminated 2 s after instruction offset triggered a reaching movement toward a lever indicated by the instruction light. Correct responses were rewarded with a drop of juice. Typical DA neuron impulses were recorded during and after task conditioning from areas A8, A9 and A10 in 2 Macaca mulatta monkeys. During conditioning, 70%, 21% and 21% of 28 neurons responded phasically to instruction stimuli, triggered stimulus and delivery of reward, respectively. Responding neurons were located throughout areas A8, A9 and A10. In all cases, responses predominated during instruction offset. After task acquisition, the fraction of the neurons responding to instruction and trigger stimulus increased (38% and 48% of 73 neurons, respectively), whereas reward responses diminished (11%). In delayed reaction tasks the reward was either given after each successful movement or movements and instructions were absent. Of 88 DA neurons, 65% and 52% responded to the trigger and reward, respectively. Trigger responses were lower before task acquisition and after overtaking, and reward responses diminished after overtaking. None of the DA neurons in either task showed delayed relation sustained activity typical for stimulus and frontal cortex.

These data demonstrate that DA neurons respond to the most significant attentional and motivation-related stimuli. During cognitive tasks, the D-specific activity does not reflect the specific cognitive components of the tasks, such as preparation of movement, expectation of external stimuli, expectation of reward, or working memory. Rather, DA neurons appear to participate in underlying attentional and motivational processes necessary for cognitive performance.

482.11 A COMPARISON OF PREMOTIONAL NEURAL ACTIVITY IN MONKEY NEOSTRIATUM AND SENSORIMOTOR CORTEX. E.J. Traynor and J.K. Carder. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

Premotion activity (PMA) of primary somatosensory (SI) and primary motor (MD) cortical neurons is correlated with the prediction of a movement, and is dependent on the learning history of the movement. However, little is known about the neural activity in sensorimotor cortex during task learning. Previous studies have shown that the activity in area 3b is correlated with movements of body parts that are controlled by the limb that generated movement. We recorded the activity of 99 neurons in SI and MD in animals trained to make wrist flexion movements. In four animals, one hemisphere was lesioned for 3 days and the other for 1.5 months, with 1.5 months of data analysis. Lesions were unilateral, and limited to the sensorimotor cortex. Activity was compared for cortical or neostriatal neurons triggered movements. Recent studies suggest that some neostriatal neurons also discharge early in similar tasks. PMA was compared for cortical or neostriatal neurons triggered movements. 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Recent studies suggest that some neostriatal neurons also discharge early in similar tasks. PMA was compared for cortical or neostriatal neurons triggered movements. Recent studies suggest that some neostriatal neurons also discharge early in similar tasks. PMA was compared for cortical or neostriatal neurons triggered movements. Recent studies suggest that some neostriatal neurons also discharge early in similar tasks. PMA was compared for cortical or neostriatal neurons triggered movements. Recent studies suggest that some neostriatal neurons also discharge early in similar tasks.

The results suggest that: (1) PMA in neostriatal neurons can occur prior to that in MD and SI, (2) unlike area 1 neuronal PMA, decreased PMA magnitude in vibratory- cue trials was not significantly correlated to area 3b neuronal PMA. Activity of MD and SI neurons could influence corticostratal neuronal PMA via demonstrated pallidothalamocortical circuitry projecting ultimately to MD and SI. Support by NIH Grant NS3437.
**BIPHASIC EFFECT OF SUBTHALAMIC NUCLEUS ACTIVITY ON SUBSTANIA NIGRA DOPAMINE NEURON FIRING. L.D. Smith and A.A. Grace. Departments of Behavioral and Cognitive Neurosciences, University of Pittsburgh, Pittsburgh, PA 15260.**

The subthalamic nucleus (STN) is known to play a major role in the regulation of movement by the basal ganglia. Both anatomical and electrophysiological studies show that the rat STN directly modulates basal ganglia output via an excitatory projection to the subthalamic nucleus (STN). Furthermore, several studies suggest that the STN also projects to dopamine (DA) neurons of the substantia nigra zona compacta (SNc), although the extent of the STN's effect on DA remains unclear. Our prior investigations have shown that the STN is involved in the induction and maintenance of burst firing in SNc DA cells. Here, however, behavioral (Bolloglou and Pagor; Brief Rev. 51, 1990) or electrical excitation of the STN rarely elicits a clear effect on DA cell activity. In this study, we examined the effects of GABA	extsubscript{A} agonist and agonist injection into the STN on SNc DA neuron firing rates.

Microinjections of the GABA	extsubscript{A} agonist bicuculline methiodide (200 μM, 200 μl) into the STN resulted in an immediate 70.3 ± 34.5% decrease in DA cell firing frequency (N=6). Although these neurons recovered their baseline firing rates within 7 to 16 minutes following the injection, there was an 81.8 ± 36.8% increase in the proportion of spikes fired in bursts. This is consistent with our finding that electrical stimulation of the STN results in a population increase in burst firing. In contrast, injection of the GABA	extsubscript{A} agonist muscimol into the STN (200 μM, 200 μl) caused a rapid and reversible 15 to 150% increase in DA neuron firing rates (N=6).

Thus the STN appears to exert a dual action on SNc DA cells: 1) an inhibition possibly mediated through STN excitation of the subthalamic SNr or globus pallidus projections to the SNc, and 2) a facilitation of burst firing which may be a direct effect of excitatory STN afferents. The activation of the STN in DA-depleted animals shown by metabolic mapping studies could contribute to the elevated level of DA neuron burst firing reported in 6-OHDA-treated rats (Hollerman and Grace, 1990).

(Supported by U54 NS31908, MH42217, MH45516 and MRC of Canada)
483.3 DIFFERENTIAL EFFECT OF SUBthalamic NUCleUS LESION on D1 and D2 AGONIST-INDUCED ROTATION IN 6-Hydroxydopamine LESIONED Rats. J.L. Anderson, T.M. Engber, and T.N. Chase, ETB, NINDS, NIH, Bethesda, MD 20892.

Evidence suggests that activity of the pathway from the striatum to the lateral globus pallidus mediated by projections to the globus pallidus pars reticulata and medial globus pallidus, as an indirect effect of nigrostriatal dopamine cell loss, may contribute to motor impairments observed in Parkinson's disease. The effect of STN lesion on D1 agonist SKF 38393 and D2 agonist quinpirole-induced rotation was examined in rats with unilateral 6-hydroxydopamine lesion of the medial forebrain bundle. Ten days following infusion with 6-hydroxydopamine, the nigrostriatal lesion was verified by examining the rotational response to apomorphine (0.05 mg/kg sc). A homogeneous group of responders was selected and half of these animals were microinjected with the neurotoxic agent quinolinic acid (60 nanomoles/0.2 ul phosphate buffered saline vehicle, pH 7.4) in the STN. The other half were microinjected with 0.2 ul vehicle in the STN and served as controls. After 7 days, the rotational response to SKF 38393 (1.5 and 5.0 mg/kg sc) and quinpirole (0.1 and 0.5 mg/kg sc) was examined. In STN-lesioned rats, both SKF 38393 and quinpirole-induced rotations were markedly reduced relative to controls in a dose-dependent manner with a significantly greater reduction seen in quinpirole turning (93% reduction) compared to SKF 38393 turning (69% reduction). These results indicate that lesion of the STN differentially affects D1 and D2 agonist-induced rotational behavior in 6-hydroxydopamine lesioned rats.

483.4 EFFECTS OF KAPPA OPIOID AGONISTS ON ROTATIONAL BEHAVIOR IN 6-HYDROXYDOPAMINE-LESIONED Rats. R.C. Bolding, T.M. Engber, and T.N. Chase, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

The effects of dopamine receptor activation in the striatum are mediated by projections to the globus pallidus and the substantia nigra pars reticulata. Recent studies have suggested that D2 receptor agonists are predominantly expressed on neurons which project to the globus pallidus while D1 receptors are predominantly localized on the nigrostriatal projections. The opioid peptide dynorphin is thought to act as a neurotransmitter in the striatogniral projection and to produce its effects by activating kappa opioid receptors. To evaluate the role of dynorphin receptor activation in motor function, we have examined the effects of the kappa agonists U50,488 (2.5, 10 and 25 mg/kg, s.c.) and spiradoline (0.5, 1 and 5 mg/kg, s.c.) on the rotational behavior elicited by D1 and D2 dopamine agonists in rats which were unilaterally lesioned with 6-hydroxydopamine. Both U50,488 and spiradoline dose-dependently decreased the rotational response to the D1 agonist SKF 38393 (1.5 mg/kg, s.c.). Conversely, neither drug affected the total number of turns produced by the D2 agonist quinpirole (0.1 mg/kg s.c.). However, changes were observed in the time-course of quinpirole-stimulated rotation following kappa agonist treatment at the highest doses tested. U50488 and spiradoline appeared to decrease the initial response to quinpirole, followed by an increase in turning in the later time periods. Taken together, these data indicate that kappa agonists produce differing effects on D1 versus D2 agonist-induced behaviors; the data also suggest that endogenous dynorphin may act to dampen striatal output through the D1 receptor-regulated striatogniral pathway.


Concomitant stimulation of both D1 and D2 subtypes of dopamine (DA) receptor is normally required for the expression of behavior, a phenomenon referred to as D1/D2 synergism. Certain treatments, however, such as destruction of D1ergic neurons and repeated treatment with reserpine induce a breakdown in synergism such that independent stimulation of either receptor subtype elicits behavior. To identify the factors involved in the breakdown of synergism, we injected rats with vehicle or reserpine (1 mg/kg) at 0, 24 and 48 hours. Two hours after the last injection, reserpine- but not vehicle-treated rats showed stereotyped motor behavior in response to quinpirole (3 mg/kg) while D1 receptors were antagonized (SCH 23390, 0.1 mg/kg), indicative of a breakdown in synergism. Rats treated with the same reserpine regimen and given L- DOPA/carbidopa (100/25 mg/kg) every 6 hours from 0 to 48 hours also showed a breakdown in synergism. Rats treated with SCH 23390 and eticlopride (0.5 mg/kg each) every 6 hours from 0 to 48 hours also showed a breakdown in synergism when tested 24 hours after the last neuroleptic injection. The results suggest that treatments that profoundly interfere with the ability of DA to interact with its receptors over the course of 48 hours result in a breakdown in synergism. The inability of L-DOPA/carbidopa to reverse this effect may be due to the failure of the intermittent injection schedule to normalize synaptic DA content.


Haloperidol is used to decrease the severity of several different hyperkinetic syndromes in humans with damage in the basal ganglia. Previous work has discovered a hyperkinetic syndrome in the rat which is induced by ventromedial striatopallidal lesions and triggered by oral sensory stimulation (Berridge et al., 1988). The hyperkinesia appears as exaggerated and perseverative forepaw treading and is never seen in intact rats.

To better characterize the neuropharmacological nature of the syndrome, systemic injections of haloperidol were given to rats expressing the paw treading syndrome. Quinolinic (QUIN) acid (60NM in .5ul) was injected bilaterally into the ventromedial striatopallidal complex. Behavioral testing began 48 hrs, post surgery. Rats were given injections (ip.) of either haloperidol (1mg/kg) or diazepam (5mg/kg) 30 minutes prior to the taste infusion. Most rats were tested with both haloperidol and with vehicle on separate days. Tests of motor abilities (wire mesh hanging time, righting reflex latency) were completed to record general motor effects of the drug injections.

Results showed that haloperidol was effective in ameliorating the hyperkinesia only in the rats that showed mild to moderate treading post-lesion and not in rats expressing severe treading post-lesion. Diazepam did not decrease the hyperkinesia and in fact, increased it in most cases. Diazepam but not haloperidol impaired performance on motor ability tests. These results implicate a role for dopamine in the expression of the hyperkinetic treading syndrome in the rat.


Rats were trained to perform a visual spatial discrimination, where stimulus information provided information regarding the required direction of response. The visual stimuli were presented either in advance of a temporally unpredictable auditory imperative stimulus (simple reaction time condition) or simultaneously with an unpredictable auditory imperative stimulus (simultaneous reaction time condition). Rats were given unilateral lesions of either the lateral or medial striatum using 1 microirre of 0.06M ibotenic acid. Following the lateral lesion an ipsilateral response bias and lengthened contralateral reaction times were seen in both conditions. In the group with the medial lesions no response bias was observed. No consistent effect on reaction time was seen following the surgery although there was an indication of contralateral reaction time lengthening in the choice condition only.

These results suggest a functional dissociation of the medial and lateral striatal regions in the control of reaction time and are relevant to studies of the effects of basal ganglia disorders on reaction time performance.


The authors of previous studies using reaction time (RT) procedure have described the effects of globus pallidus (GP) lesion on the performance of an arm movement in response to a visual stimulus. So change in the RT was reported in any of this result of GP lesion. These results suggested that the GP was not involved in motor initiation. This conclusion was not in agreement however with some electrophysiological data showing the existence of GP cells which discharge before the onset of the movement. In our study, we investigated the effects of GP lesion on both the simple reaction time (RTs) and the choice reaction time (RCTs) in a pointing task with variable movement amplitude. Two monkeys were trained to perform the RT paradigm. After unilateral neurochemical GP lesion, no change in the RTs was observed, whereas a significant increase in the RTs occurred in the contralateral limb. These results indicate that the GP may in involved in motor initiation in RTc task particularly when there is uncertainty as to the amplitude of the forthcoming movement.
483.9 TEMOROUS ORAL MOVEMENTS AND FEEDING DEFICITS IN RATS WITH VENTROLATERAL STRIATAL DOPAMINE DEPLETIONS: POSSIBLE RELATION TO PARKINSONIAN SYMPTOMS. J. D. Salome, G. Jicha, and S. Rogers. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.
Local depletion of dopamine (DA) produced by injections of 6-hydroxydopamine in the ventrolateral striatum (VL5) caused a substantial decrease in food intake, from which the animals recovered. In behavioral observation sessions, rats with VL5 DA depletions showed no reduction in time spent feeding, but did have substantial impairments in the rate of feeding and forepaw use during feeding. DA depletions in nucleus accumbens, anteroventral caudate (A11V) or dorsolateral striatum (DLS) did not produce significant feeding deficits. Rate with VS DA depletions showed no deficits in locomotor activity or rearing. Tremulous chewing movements were produced by VL5 DA depletion, but not A11V or DLS depletions. Systemic administration of haloperidol (0.4 mg/kg) increased tremulous chewing responses in DA-depleted and control rats. Thus tremulous chewing responses can result from reduced functional activity of VL5 DA, and these responses share some characteristics with human Parkinsonian symptoms. These data support the notion that the neostriatum is functionally heterogeneous and that the VLS is particularly important for oral and forelimb motor control.

Disruption of nigrostriatal inhibition results in saccadic jerks during fixation and saccades with shorter reaction time (RT), higher peak velocity (PV) but smaller amplitude (MAMP), especially to pre-fixed targets (Hikosaka and Wurtz). We examined the effect on arm movements produced by changing the output of the globus pallidus (GPi). Monkeys were trained to make arm movements to targets illuminated coincident with a GO tone or were lit briefly as a pre- cue. Injection of muscimol into GPi produced a flexor drift of the contralateral arm from the start position. When the initial position was maintained until the GO tone, the RT was normal, but the MAMP and PV to some targets were decreased and the amplitude/peak velocity scaling relation was disrupted. Injection of bicuculline into GPi did not cause a flexor drift, but it produced involuntary movements, primarily proximally. RTs were not changed, but the MAMP and PV also were reduced to targets in some directions. Positional instability of the eye or arm follows injections of muscimol into appropriate basal ganglia nuclei. However, changes in arm movements produced by changes in GPi output were more complex than predicted from oculomotor studies.

The purpose of this investigation was to determine whether D1 and D2 dopamine receptor subtypes have different roles in modulating the speed and success of rodent reaction time performance. Animals were shaped to release a lever in response to an auditory/visual stimulus in order to avoid mild footshock. Shaping was continued until the animals were responding reliably with response latencies of 180-220 msec. The effects of apomorphine (0.15, 30, 60, 1.2, 2.4 and 4.8 mg/kg) on reaction time were determined in animals pretreated with the selective D1 antagonist, SCH 23390 (0, 50 and 100 μg/kg), or the selective D2 antagonist, spiperone (0, 1 and 10 μg/kg). Apomorphine alone resulted in a dose dependent decrease in successful avoidance with an ED50 of 3.9 mg/kg. Response latencies were unaffected by apomorphine treatment. SCH 23390 pretreatment resulted in a significant impairment of both successful avoidance and in some latency measures. Increasing doses of apomorphine effectively attenuated SCH 23390's effects on successful avoidance, and to a lesser extent blocked the SCH 23390-induced increase in response latency. Spiperone pretreatment attenuated the effects of apomorphine on successful avoidance, resulting in a 6-fold shift to the right in the apomorphine dose-response curve. These results suggest that D1 receptors play an important role in modulating the speed of reaction time responses and that D2 receptors predominantly modulate successful reaction initiation.

483.13 ORIENTATION TO EDGES AND ITS MODULATION BY STRIATAL DOPAMINE IN RATS. R. Sullivan, A. Fraser, C. Coulier and R. Stephan, Dept. Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada L8S 3J5.
Male Sprague-Dawley rats received either control operations (N=14) or unilateral 6-hydroxydopamine lesions of the left or right substantia nigra (N=22 per group). At eight weeks post-lesion, all rats were tested (undrugged) in a large open field (160 cm square, 100 cm high) for ten minutes. From videotapes, a measure of set orientation was obtained for time spent at the edge (locomotor activity). Following testing, brain regions were assayed for levels of DA and metabolies by HPLC. Left and right lesioned rats differed significantly in their orientation (p<0.001). Following treatment with d-amphetamine (0.01 mg/kg), the intact striatum was contralateral to the edge. When controls were subdivided according to their left/right asymmetry in DA turnover, it was found that the intact DA activity being contralateral to the edge, DA activity in other regions was unrelated to edge behavior, emphasizing the importance of striatal DA in directing behavior. Because attraction to, or exploration of edges appear to be regulated at behavioral levels, these results suggest that edges are significant stimuli in the organization of behavior. Supported by NSERC.

483.14 INVOLVEMENT OF GLUTAMATE RECEPTORS IN THE ZONA INCERTA IN AMPHETAMINE-INDUCED STEROTYPY BUT NOT LOMOCOMOTOR ACTIVITY. R.K. Supko, R.J. Uretsky, J.L. Wallace. College of Pharmacy, The Ohio State University, Columbus, OH 43210.
A pathway projecting from the nucleus accumbens (NA) to the subpallidum and on to the pedunculopontine nucleus (PPN) has a role in locomotor activity (LMA). This role is likely mediated by non-NMDA receptors. In order to determine whether this role is also mediated by glutamate receptors, we have investigated the involvement of non-NMDA receptors in the effects of amphetamine on LMA.

In order to determine whether this role is also mediated by glutamate receptors, we have investigated the involvement of non-NMDA receptors in the effects of amphetamine on LMA.

We have found that the amphetamine-induced hyperlocomotion in the ZI is mediated by the NMDA receptors. In order to determine whether this role is also mediated by glutamate receptors, we have investigated the involvement of non-NMDA receptors in the effects of amphetamine on LMA.

We have found that the amphetamine-induced hyperlocomotion in the ZI is mediated by the NMDA receptors. Additional experiments have revealed that the ZI is involved in the amphetamine-induced hyperlocomotion, and that the amphetamine-induced hyperlocomotion is mediated by the NMDA receptors.

The results of these experiments suggest that the ZI is involved in the amphetamine-induced hyperlocomotion, and that the amphetamine-induced hyperlocomotion is mediated by the NMDA receptors.

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483.15

DOXAMINERGIC INPUT TO THE SUBTHALAMIC NUCLEUS REGULATES LOMOTOR ACTIVITY IN THE PARKINSONIAN RAT. G. Flores*, D. Martinez-Fong and J. Aceves, Dept. of Physiology, CINVESTAV-IPN, Mexico.

Here we studied the role of the DA innervation of the subthalamic nucleus on locomotor activity (loc act.). 6-OHDA was injected bilaterally into the pars compacta. Shams: saline. Injected site was assessed histologically. DA content was measured in striatum (st), accumbens (acc) and subthalamic nucleus (STN). Two groups of rats could be distinguished, one with decreased (from 23 ± 2 to 7 ± 2 counts/10 min), and another with increased (from 23 ± 2 to 69 ± 2) loc act. No change (before: 23 ± 2; after: 24 ± 2) in loc act was seen in sham injected sites in hypokinetic rats rostrally, and in hyperkinetics ones they were significantly located in pars compacta. DA (ng/mg prot.) contents were (mean ± SD; n = 6):

- Nucleus hypokin. hyperkin. shan
  - Str 3.7±1.0 4.6±0.8 10±0.4
  - acc 27±5 16±5 62±3
  - STN 0.6±0.1 3.7±0.2 3.7±0.2

Hypokinesia was associated with loss of DA input to subthalamus. Data suggest that STN is normally under a tonic inhibitory DA action via a nigrosubthamic pathway. Loss of this action, disinhibits the STN, producing hypokinesia (or akinnesia).

483.17

IN VIVO SUBREGIONAL EFFECTS OF AMPHETAMINE AND CORTICAL STIMULATION IN THE RAT CAUDATE PUTAMEN. G.E. GLYNN and B.K. YAMAMOTO, Northwestern Ohio Universities College of Medicine, Rootstown, Ohio 44263.

A topographic projection from the cortex to the striatum exists in the rat. The effects of dopamine on the electrophysiology of this corticostriatal projection are controversial. The purpose of this study was therefore to determine the relative electro-physiological importance of these projections and the regional dopaminergic inputs that play into modulating this striatal input.

An 8 electrode array of graph-epoxy volumetric electrodes, each electrode having a multiplex unit activity (MUA) and dopamine, was placed at two depths in the striatum. 8 cortical sites were repeatedly stimulated. Amphetamine was administered and its effects on dopamine electro-physiology were monitored. Overall there was a 100% greater dopamine concentration increase in dorsolateral as compared to dorsomedial regions, a 52% greater increase in ventrolateral as compared to ventromedial and a 56% greater increase in dorsal as compared to ventral region of the STN. A distinct region pattern in the striatal electrophysiological response to each cortical stimulation site was evident. Data will be presented on the effects of dopamine concentration changes on this electrophysiology response within 14 subregions of the stratum.

484.1


In an effort to characterize length-tension relations in spastic muscle, torques produced over a range of elbow angles in the impaired versus unimpaired upper extremity were compared over a range of elbow angles in the impaired versus unimpaired upper extremity. This may be caused by increased compliance of the series elastic element, increased tendon length, changes in fiber length, or other adaptations of the muscle-tendon complex. This work is supported by NIH grant NS-19331.

484.2


Is more masseter (MA) activity required to generate a given force when biting on incisors than when biting on molars? To answer this question we built splints for each of 4 subjects (Ss) to keep jaw separation at 10 mm and MA length constant whether the bite-transducer was between molars, cuspids or incisors. Fine wires in the right MA detected unit activity. Ss generated clenches of 15, 30 or 60 N while viewing the transducer output. From continuous recordings, relationships between firing rates and forces were determined for each bite location and clenched strength. Interspike intervals (ISIs) were shorter for any given force when clenching on incisors than on molars. A 20-msec delay in ISIs was associated with clenching on incisors than on molars. Increasing bite force lead to recruitment of new units and a decreased mean ISI in every unit. We conclude that to maintain the same force at the incisors as at the molars more MA activity is required.
4.4.3 EFFECTS OF DORSAL COLUMN LESIONS ON MOTOR CONTROL AND LONG-LATENCY RESPONSES IN THE INDEX FINGER OF MACAQUES D.S. Gerdienbrock and C.E. Videoak, Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

These experiments were designed to test the hypotheses thatafferents carried in the dorsal columns (DCs) 1) are needed to regulate the forces of finger movement and 2) form the after-discharge of long-latency stretch reflexes. Three stump-tailed macaques were trained to hold the index finger in a fixed position during voluntary movements of the elbow joint in order to induce a "force" sensation. ("move and hold") or a "fixed" joint condition ("move and hold") or a "move" condition. The after-discharge was elicited by a sudden stretch of the elbow joint, while the monkeys were instructed to "move" the joint in order to generate a "force" sensation. The after-discharge was monitored using electromyographic (EMG) activity. The results showed that the after-discharge was more pronounced in the "move" condition and was accompanied by increased EMG activity. This suggests that the dorsal columns play a role in the regulation of motor control and long-latency responses in the index finger of macaques.

4.4.4 EFFECTS OF ALTERING VISUAL AND PROPRIOCEPTIVE INFORMATION DURING VISUOMOTOR TRACKING. S.H. Brown, J.D. Cooke, T. Ohtsuki.


Locomotion can be induced by electrical stimulation of the mesencephalic locomotor region (MLR) in the decerebrate cat. The MLR has been shown to project to the spinal cord via the pontomedullary reticular formation (Noga et al., J. Neurosci., 1987) where it is thought to produce locomotion by activation of interneurons in the spinal cord (Dai et al., Soc. Neurosci. Abstr., 1986). In this study, we investigated the effect of task intent on the kinematics of the free motion phase when grasping for different shaped dowels. The results showed that the kinematics of the free motion phase when grasping for different shaped dowels was influenced by task intent. The dowel shape affected the motion of the arm and hand during prehension, and may influence the selection of motor strategies.


Spatio-temporal analyses of a marker placed on the wrist (transport kinematics) have been the result of using a dowel with square gripping plates, since Marteniuk et al. (1987) have shown that this method was more effective in generating kinematic variables in the free motion phase when the arm and hand were quite close to the target. These findings suggest that dowel shape does influence the kinematics of the free motion phase and may be a factor in the selection of motor strategies.

4.4.6 SPINAL INTERNEURONS SYNAPTICALLY ACTIVATED BY STIMULI APPLIED TO THE MESENCEPHALIC LOCOMOTOR REGION. L. MacKenzie and B.R. Noga. Department of Physiology, University of Manitoba, Winnipeg, Man., Canada, R3E 0W3.

Few lumbar spinal interneurons have been described which show activation by stimuli applied to the mesencephalic locomotor region (MLR). In this study, we investigated the effect of task intent on the kinematics of the free motion phase when grasping for different shaped dowels. The results showed that the kinematics of the free motion phase when grasping for different shaped dowels was influenced by task intent. The dowel shape affected the motion of the arm and hand during prehension, and may influence the selection of motor strategies.


Weir and MacKenzie (1991) examined the effects of task intent on the kinematics of prehension when reaching for a dowel that had been instrumented for quantifying grasping forces. While the grasping forces were found to be appropriate for the different tasks, the after-discharge of the different tasks was found to be different. The after-discharge was found to be more pronounced in the "move" condition and was accompanied by increased EMG activity. This suggests that the dorsal columns play a role in the regulation of motor control and long-latency responses in the index finger of macaques.

4.4.8 MOVEMENT VARIABILITY ASSOCIATED WITH DIFFERENT SHAPED DOWELS. C.L. MacKenzie and P.L. Weir. Dept. of Kinesiology, University of Waterloo, Canada, N2L 3G1.

Movement variability associated with different shaped dowels was found to be different. The variability was found to be influenced by the ability of the dowel to generate a "force" sensation. The variability was found to be greater when the dowel had a flat, square gripping surface, while the other dowel (the "cylindrical" dowel) had a curved gripping surface. The variability was found to be greater when the arm and hand were quite close to the dowel. These results suggest an influence of dowel shape on variability measures associated with both the arm and hand, reflecting the tight coupling between the two components. (Supported by NSERC)
484.9

Electromyographic (EMG) analyses have been used to demonstrate that a common muscle synergy may serve to regulate stance posture in the presence of either voluntary or external perturbations (Frank and Earl, 1991). EMG analyses reflect the gross motor unit recruitment, but kinesthetic and kinetic data are required to predict the body movements which stabilize stance. The present study was to conduct an inverse dynamic analysis of the compensatory strategies used to cope with external and voluntary perturbations. Subjects were instructed to maintain position against a 20 N background load and were perturbed by a step increase in either the handle force (100 N, 300 ms) or an anterior background load (20 N background load, acting in the anterior direction). Perturbations were created when subjects pulled the handle against the load. External perturbations were generated by imposing a step change in the handle force (100 N, 300 ms) either forward or backward. The latency and magnitude of biphasic ground reaction force responses and EMG data were used to delineate changes in the ankle, knee and hip joints. Anterior displacement of the center of pressure observed for the external perturbations exceeded the magnitude of those observed during voluntary perturbations. Net joint moments varied both between and within subjects. The pattern of muscle activation acting about a joint; joint power indicates the energy absorption and generation which occurs at a point. Invariant compensatory actions, as illustrated by the net joint moments, were used to cope with both types of perturbations: predominant ankle plantarflexor moments were accompanied by smaller knee flexor and hip extensor moments. Joint power profiles revealed energy absorption at the ankle; between and within subject differences in activation and generation were observed as the knee and hip joints. 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Galloping at moderately high treadmill speeds (3-4 m/s) is difficult to elicit in the cat and has not been well assessed. We trained cats to gallop at these speeds and quantified hindlimb dynamics (for methods see Smith and Zemekic, TINS 10: 123-128, 1987) and EMG of selected muscles (see Buford and Smith, J. Neurophysiol. 57: 756-766, 1987). We focus here on hindlimb dynamics during the swing phase (65%) of the step cycle and action requirements for bipedal postural thoracic muscles, such as semimembranosus (ST), that extend the hip and knee and act as antagonists to semitendinosus (SF) and gastrocnemius (LG), that flex the hip and extend the knee.

At the hip joint in early swing, an extensor muscle torque is needed to counterbalance a large flexor torque created by leg angular acceleration (LAA). But, during the walk and trot, active muscles provide a flexor torque and there is no need for an extensor muscle torque. LAA creates an extensor torque. In early swing, therefore, muscles that flex the hip and extend the knee must counter LAA; both requirements can be satisfied by activation of the BF or SF.

Later in swing, passive muscle forces appear responsible for decelerating forward motion of the limb associated with LAA, because muscles with hip extension and/or knee extension functions are not active before hip extension and knee flexion begin. During the walk, active muscle torque from posterior thoracic muscles (e.g., ST) decelerates forward limb motion prior to paw contact, but during the gallop, ST activity occurs just as or after knee flexion begins and is associated with rapid knee flexion and hip extension prior to paw contact. Our results suggest that swing phase limb dynamics during the gallop, in contrast to the walk and trot, require different demands of active bipedal muscle. These changes have not been considered by those who emphasize only the need to change interlimb coordination to control the gait. Research supported by NIH NS 19864.

The hypothesis of a mental rotation of a movement vector when a movement has to be made at an angle from a stimulus direction is supported by data on behavioral (Georgopoulos et al., Exp. Brain Res. 65:361, 1987) and neural studies (Georgopoulos et al., Science 243:234, 1989). Does this process share common constraints with the mental rotation of visual images (Shepard & Cooper, Mental Images and their Transformations, 1982)? To answer this question, we took advantage of the fact that mental rotation rates (reaction times) may vary considerably among subjects: If common constraints underlie the spatial transformations involved in these tasks, then a correlation should be observed between the rates of mentally rotating visual images and those of mentally rotating a movement vector. We found that, indeed, these rates were significantly correlated (r=0.001, N = 39 naive human subjects); in contrast, mental motor rotation rates did not correlate with rates (reaction time vs. number of movements in a memorized list) observed in a context-recall visuomotor memory scanning task (Georgopoulos & Lurito, Exp. Brain Res. 83:453, 1991) which does not involve mental rotation. These results suggest that perceptual and visuomotor mental rotation tasks possess common processing constraints. (Supported by NIH, ONR and HSPF.)


Soechting & Terzuolo have shown (Neuroscience 23:39, 1987) that the plane of movement of the wrist in free space may change abruptly between segments of the trajectory and be almost constant within each segment; this change of plane occurs in the proximal parts of the trajectory and may be a consequence of a segmentation at the level of motor programming. It was also shown that, for a given plane, the phase relations among upper- and fore-arm angular elevation are kept constant, which suggests that they can be part of an internal coordinate system for planning of movement trajectory. It can then be worth to analyze the plane of force trajectory in the absence of overt joint motion. In our experiment, subjects exerted forces with an unstrained arm and the hand pronated on a 3-D isometric handle which controlled the position of a visual cursor in a stereographic display (Massey et al., J. Neurosci. Methods 26:123,1988). They were asked to trace lemniscates (figures "B") in frontal or sagittal planes. We noticed that the planar orientation of the force trajectory often changed by more than 20 degrees between the two loops of the lemniscate, which by definition are drawn with centers on the same plane of motion.

The neuromuscular system of Ascaris musculus consists of longitudinally oriented muscle cells that give off processes to contact the nerve cords. The prolongations from many cells intertine at the surface of the cords to form the muscle syncytium or sarcopile. At this level the muscle cells exhibit myogenic activity, forming neuro-muscular junctions and are coupled by gap junctions. The possible role of gap junctions in contractile wave propagation is not yet known. Our data from scanning microscopy of the syncytium show the arms approaching the nerve cord in bundles and each bundle corresponds to arms from one or two adjacent rows of cells. Intracellular recordings show that the electrical coupling between cells is more efficient in narrow circular bands perpendicular to the longitudinal axis than along the length of the animal. We suggest that synchronization of electrical activity and contractile wave propagation is due in part to sequential activation of well coupled functional units of contraction (cell rows) that are perpendicular to the longitudinal axis. Thus, longitudinally oriented cells may present circular patterns of contraction as a consequence of the gap junction coupling between them.

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485.3 THE CONTRIBUTION OF VISUAL INTERNEURONS TO DIRECTION-SELECTIVE RESPONSES OF A FLIGHT MUSCLE. J.J. Milde, II. Zoologisches Institut, Universität of Cologne, Weyertal 115–3000 Köln 41, F.R.G.

In the sphinx moth Manduca sexta, appropriate visual stimuli evoke compensatory body and limb movements. Intracellular staining and recordings demonstrate that numerous brain interneurons responding to optomotor stimulation are present in the optic lobes (medulla, lobula and lobula plate) as well as in the posterior midbrain or the protocerebral bridge. To find out whether a recorded interneuron really participates in behavior or not requires a simultaneous analysis of neuronal and motor responses.

In this study, the tonic unit of the mesothoracic third auxiliary muscle [Rheuben MB, Kammere AE (1987) J exp Biol 131:373] has been shown to respond to visual stimuli even in intact animals. Synaptic processes on the cell bodies of these brain interneurons responding to optomotor stimulation are present in the optic lobes (medulla, lobula and lobula plate) as well as in the posterior midbrain or the protocerebral bridge. To find out whether a recorded interneuron really participates in behavior or not requires a simultaneous analysis of neuronal and motor responses. Intracellular recordings were obtained from the medulla, lobula and lobula plate. The contribution of an interneuron to the motor activity is further evaluated by current injections. For example, a lobula plate neuron, which corresponds well with the muscle activity if the direction-selective responses are compared, can be shown by hyperpolarization to be obviously not involved in the generation of the muscle activity.

This approach is an important step towards an identification of neuronal elements underlying visually guided behavior.

485.4 SEGMENT-SPECIFIC FATES OF PROLEG MUSCLES AND MOTONEURONS DURING METAMORPHOSIS IN MANDECA SEXTA. D.J. Sandstrom and J.C. Weeks. Graduate Group in Neurobiology, Univ. of Ca., Berkeley, CA 94720, and Inst. of Neurosciences, Univ. of Oregon, Eugene, OR 97403.

The abdominal prolegs of Manduca are larval locomotor appendages that are lost at pupation. In the proleg-bearing segments, A3-A6, the proleg motoneurons (MN) innervate the larval proleg retractable muscle, APM. Proleg homologs of the APMs are also present in the non-proleg-bearing segments, A1 and A2. To better interpret other experiments investigating metamorphic changes in synaptic inputs to the APMs [J. Comp. Neurol. (1991) 308:311], we have examined these MNs and muscles in more detail. In larve, each APM is innervated by a pair of APMs, which appear to be identified by electrophysiological and anatomical criteria. Typically, the anterior fibers of an APM are innervated by one APM and the posterior fibers by the other APM, with middle fibers being dually innervated. Synaptic potentials from the two APMs have similar amplitudes and shapes, and exhibit similar activity-dependent plasticity. During the larval-pupal transformation, the APMs degenerate in all segments except A2 and A3, whereas the APMs die one day after pupation in all segments except A2, A3 and A4.

The denticular arbors of APMs in the different segments resemble a similar extent regardless of whether the APM, or its target muscle, is killed to die. The surviving APMs and APMs persist through the pupal stage, with the APMs showing dendritic growths. During the first two days of adult life the APMs and APMs degenerate, presumably after participating in adult emergence behaviors. Thus, the degeneration of the APM and APM is segment-specific, while the dendritic regression of the APMs is not.

Supported by the NIH and NSF.


Larval leg motoneurons (MN) in Manduca persist to innervate the new adult legs. We are interested in the intrinsic properties of these MNs that may be important for locomotor activity. Using whole-cell patch-clamp techniques, we have found that the MNs express two types of putative Ca2+ currents: the dominant current has a low-threshold (40 mV) and is slowly inactivating, the smaller one has a higher threshold and is resistant to inactivation (Levine and Hayashi, J. Neurophysiol. 65:351-363). These Ca2+ currents reflect properties expressed in situ, intracellular recordings were obtained from leg MNs in intact ganglia. Following perfusion with saline containing TTX and TEA, plateau-like potentials could be evoked with short depolarizing pulses and suppressed at more depolarizing offsets. Two components of the plateau-like potentials were distinguished: (1) transient, large amplitude potentials evoked at negative offsets and suppressed at more depolarizing offsets, (2) smaller, persistent potentials activated from depolarizing offsets. The plateau-like potentials occurred only in the presence of TEA and disappeared following perfusion with normal saline. They were TTX-insensitive, with Ba2+ and Co2+ and suppressed with Ca2+ and Ni2+.

The significance of these potentials for normal function is unclear, but they may augment rhythmic synaptic input during locomotion, perhaps after being revealed by neuromodulatory substances.


Previous investigations have shown that the flight motor pattern of the locust relies heavily on activity in the hindwing tegula but not on activity in the forewing tegula. Removal of the hindwing tegula results in loss of the tegula. Recovery. These physiological changes were paralleled by sprouting of the APMs and APMs persist through the pupal stage, with the APMs showing dendritic growths. During the first two days of adult life the APMs and APMs degenerate, presumably after participating in adult emergence behaviors. Thus, the degeneration of the APM and APM is segment-specific, while the dendritic regression of the APMs is not.

Supported by grants from the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.
485.7 THE PROBLEM OF RELIABILITY IN INSECT NEURONMUSCULAR CONTROL.  
Jim H. Belanger and Ian Orchard. Department of Zoology, University of Toronto,  
Toronto, Ontario, Canada MSS 1A1.

The force which a muscle is capable of generating is proportional to its cross- 
sectional area, and the maximum force that can be sustained is proportional to the  
volume of the organism. This means that, compared to vertebrates, insect muscles  
are relatively "oversized". It also implies that, in order to achieve a given level of  
muscle force (or tension), the insect neuromuscular system must exercise a greater  
degree of control over its output (motoneuronal signalling). We wanted to discover.  
1. How reliably does a given insect neuromuscular system transduce a given neural  
input into muscle force? 2. What roles do the CNS and insect proprioceptors play in  
controlling muscle activity?

We approached this question using an in vitro preparation we have developed  
based on a whole body dissected from Periplaneta americana. The body was then  
immobilized, and a nerve was dissected out which innervated the target muscle.  
This preparation allows simultaneous monitoring of motoneuronal output, muscle  
electrical activity, and muscle force generation while the muscles are being driven  
spontaneously by a central pattern generator located in the terminal abdominal  
ganglion (K.J. Thompson, J. Exp. Biol. 122:387, 1986). In spontaneously active preparations, EMG activity is significantly correlated with  
nervinal output, but not of these is correlated with the force produced by the muscle.  
When the spontaneous input is removed (by removing the ganglion), electrical  
stimulation of the opener nerve reliably predicts both the EMG and the force  
produced by the muscle, but only if simple impulses are given. If trains of pulses  
are given, the summed EMGs are not significantly correlated with muscle force,  
even when the same population of axons is recruited with every stimulus.

We are currently examining the possibility that proprioceptors act to reduce this  
variability between axonal input and muscle output.

485.8 MOTOR PATTERNS IN COCKROACH LEGS DURING WALKING ON A SLIPPERY SURFACE.  
F. Delcomyn. Department of Entomology and Neuroscience Program,  
University of Illinois, Urbana, IL 61801.

Intact cockroaches (Periplaneta americana) show a stereotyped pattern of activity in leg muscles during free  
walking. After amputation of a leg, free-walking preparations show a pattern of motor activity that differs from normal mainly in  
the shifted timing of bursts in the leg in front of the amputated one during slow walking (Delcomyn, J. Exp. Biol. 156, 483-  
517). When suspended intact insects walk on a slippery surface, the pattern of motor activity is like that shown by  
intact insects walking freely. Amputation of a leg in suspended insects produces a motor pattern somewhat similar to that  
seen in free-walking amputee insects. However, this pattern has a reduced shift of timing of bursts in  
the leg in front of the amputated one, and a strong shift in  
the timing of bursts in the leg behind it during slow walking. The differences between the motor patterns seen in free-walking  
and suspended amputee insects are likely due to the  
mechanical uncoupling of the legs during suspended  
walking. It is inferred that sensory feedback  
from leg mechanoreceptors is normally used by the leg of origin as well as by adjacent legs to coordinate motor activity.  
Supported by a grant from the Whitfield Foundation.

485.9 NEURAL ACTIVITIES OF THE MUSCULAR BODIES OF THE  
COCKROACH DURING LOCOMOTORY BEHAVIOR.  
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Tucson, Arizona 85721.

In insects, two distinct sensory pathways that serve a motor descending  
neurons, one via deuocerebral interneurons, the other via higher centers (muscle bodies, MBs) in the prothoracic. In 1966, Huber (Z. Verg.  
Physiol. 49) demonstrated that MBs control complex behavioral reperatures. We have refined experimental  
techniques to re-examine this hypothesis.

Neural activities from MB neurons of the cockroach, Periplaneta americana, were recorded while animals were walking freely, using wire  
and recording and staining techniques which reveal intact neurons at the  
Tactile stimulations were used to induce locomotor behaviors. Units from  
MBs were classified into (1) sensory units responding to mechanical  
stimulation of the body, (2) arousal units which exhibit long-lasting bursts after  
tactile stimulation, (3) locomotory units. Units recorded at MB input  
regions (the calyces) of MBs were exclusively sensory, whereas output  
regions (pedunculi and lobes) were characterized by arousal and  
locomotory units. Focal electric stimulations at the pedunculi and lobes  
induced complex sequence sequences.

Although MBs are clearly indicated in spatial memory processing (see accompanying abstract, Welbrecht et al, 1991), and although lesions of MBs  
percurh this property, bilateral lesions of MBs do not percurh spontaneous  
motor actions. This suggests that motor activity per se can be independent  
of the participation of MBs and that MBs may specifically govern  
acquisitive motor reperatures. Supported by NIH grant F05 TW 04390.

485.11 STRUCTURE OF OUTPUT NEURONS OF THE MUSCULAR BODIES IN  
HONEYBEE BRAINS.  B.A. Harley, W. Gronenberg, and N.J. Strausfeld,  
Arizona Research Laboratories, Division of Neurobiology, University of Arizona,  
Tucson, AZ, 85721 USA.

Muscle bodies (MBs) are protocerebral neuropils implicated in learning  
Each MB comprises a pair of calyces supplying a pedunculus branching into an α  
and β lobe. Palladines of K-cells in the calyces receive a distributed differential representation of olfactory, visual, and  
mechanosensory centers. This arrangement has been interpreted as reflecting multimodal sensory integration at K-cells (Mobbs PG (1982) Phil Trans Roy Soc Lond B 298:309-354) whose axons go on to supply the pedunculus and lobes below (Harley, 1994).  
K-cells are known for the connections of the pathways and targets of pedunculus and α β lobe outputs.

The structure, arrangement, and targets of neurons leaving the α and β lobes has now been studied using a modified Golgi technique and  
histology. Serially sectioned neurons were reconstructed using camera lucida at high power. In each α β lobe, output neurons ("extrinsic neurons"; ENs)  
have elaborate dendritic arborizations characterized by specific  
configurations of the many thousands of parallel K-cell axons. Most EN axons  
estend outside the α β lobes and terminate in specific promoter nuclei of the  
deuocerebral complex. However, a substantial population of ENs project back to the  
K-cells, comprising a "feedback" loop. Such feedback supply may be important  
for memory acquisition, permitting information processed by the lobes to cycle  
back to the fusimotor (K-cells) for further processing (Schärer, 1974) Exp Brain Res 19:406-432. Supported by the Center for Insect Science  
(NSF DIR 82-20002) and NSF BNS 9011012.

485.12 THE MODULATION OF WING PHASE DURING TURNING RESPONSES OF  
DROSOPHILA. M.H. Dickinson. Dep. of Organismal Biol. and  
Anatomy, Univ. of Chicago, Chicago, IL 60637.

The turning response of Drosophila to visual stimuli consists of several behavioral components including  
abdominal steering, leg position, and alteration of wing- 
beat amplitude. Here I report an additional mechanism:  
the modulation of wing phase. At the end of the downstroke flies rapidly rotate their wings  
before initiation of the upstroke. This rapid rota- 
tion is termed the ventral reversal. The timing of the  
ventral reversal can be precisely measured using pairs of  
photo-diodes (Gözt, 1987). During optic stimulation in the  
open loop condition, flies actively modulate the timing of the  
ventral reversal such that the wing on the outside of the turn is in advance of the wing on the inside of the  
turn. The amplitude of the wing phase modulation is on the  
order of 5°. The phase response is much greater during  
imulated stimulation with a single vertical black stripe (figure),  
than with oscillation of a large field pattern (ground).  
Wing phase modulation is greatest during oscillation  
frequencies near 1 Hz.

This work was kindly supported by the Max Planck Institute  
for Biological Cybernetics.

HYPOTHALAMUS II

486.1 DIVERGENT AXON COLLATERALS ORIGINATING IN THE RAT VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. S. Trump, C. Ullens, and T. Akason. Department of Veterinary College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Cells of the ventrolateral part of the ventromedial nucleus of the rat (VMHvl) are perhaps the best characterized of neurons that influence female sexual behavior. Lesioning the VMH vl prevents and estrogen stimulates lordosis behavior. In addition to having their own estrogen receptors, neurons of the VMHvl also project to other steroid target nuclei, such as the medial preoptic nucleus (MPO), the medial preoptic nucleus of the amygdala (MeA), and the mediobrain pedunculated gray (PAG). One way in which a response to estrogen could be amplified is if estrogen-sensitive neurons send collaterals to other nuclei involved in a particular function. This possibility prompted us to test for the presence of collateral projections by neurons of the VMHvl.

Axons were labeled with Fluoro-Gold (FG) in the VMH vl and analyzed to determine whether there were axonal collaterals that projected from the VMH vl to the MeA or the PAG. FG-labeled neurons were found in the VMH vl and these neurons were found to project to the MeA or the PAG.


The paraventricular nucleus (PVN) is a key part of the hypothalamus that regulates the release of hormones that control various bodily functions. It has been shown that the PVN plays a crucial role in the coordination of autonomic functions and the regulation of the endocrine system.

The present study aimed to examine the subpopulation of PVN neurons that project to the lower lumbar spinal cord and concentrate 3H-endostriol, a steroid hormone that is involved in the regulation of various physiological processes. The study used a combination of anatomical and physiological methods to identify and characterize these neurons.

The results of the study showed that a specific subpopulation of PVN neurons, which project to the lower lumbar spinal cord, concentrate 3H-endostriol, indicating that these neurons play a crucial role in the regulation of endocrine and autonomic functions. These findings have important implications for our understanding of the neural mechanisms underlying the regulation of endocrine functions and highlight the importance of further research in this area.
486.5 PRESENCE OF CALBINDIN (CB) AND LACK OF PARVALBUMIN (PV) IN PROGESTERONE RECEPTOR-CONTAINING (PR) NEURONS OF THE PRIMATE HYPOTHALAMUS. E. Meelis*, M.R. Kruk*, and J.G. Veening*. Dept. of Obstetrics & Gynecology, Yale University, School of Medicine, New Haven, CT 06510

In primates progestanes (P) decreases the frequency of lactating hormone (LH) pulses. LH pulses are not suppressed by LH. The results suggest that P acts indirectly on the LH system. In the primate hypothalamus a large number of neurones contain PR and all of these cells are GABAergic (Kruk et al., 1990). A population of GABA neurones contains calbindin binding protein. In order to further characterize the PR-containing GABA cells we searched for their calcium-binding proteins. (Experimental) Three out of 4 adult female rats were run for 2 h after stimulation. Rats stimulated in the DMH had a similar response to control situations. A brain cannula system for chemical brain stimulation for detecting the in vivo activity was designed from a previous study. A detailed analysis was made of grooming behaviour elicited by microinjections into the hypothalamic paraventricular nucleus and adjacent dorsal hypothalamic area with glutamic acid on the hypothalamic paraventricular nucleus and adjacent dorsal hypothalamic area with glutamic acid antagonist. Increases in boutlength and frequency of bouts were observed. Decreases of the elicited grooming were similar to control situations. Blood flow in a social environment. Microinjections into the ventromedial nucleus (VMN) were performed in about 40 rats. From all origins, mentioned above, fibers are present in the ventromedial hypothalamic nucleus (VMH), ventromedial hypothalamic area (VMA), lateral hypothalamic area (LHA), and the adjoining nucleus of the lateral hypothalamic area (LHA). The hyperactivity usually persists for 2-3 hours and the magnitude of this phenomenon is often quite impressive. The mechanism underlying the locomotor activity may involve the non-specific release of dopamine at the target structures of damaged meninges and/or mesolimbic axons which traverse the lesion site. In order to test this hypothesis we anesthetized rats with haloperidol and observed this postoperative activity in both an open field in and photocell activity cages following injections of the dopamine antagonist haloperidol (1.0 or 2.0 mg/kg, sc). Neither dose of haloperidol blocked the activity, although at the higher dose some jumping was observed, which, however, was completely suppressed by mCPP.  


Descending projections have been studied in about 40 rats, with small injections of PHA-L in different parts of the hypothalamus. Projective cell populations (PVN), dorsomedial nucleus (DMH), rostromedial and caudolateral parts of the ventromedial nucleus (VMH), intermediate hypothalamic area (IHA), ventrolateral and lateral hypothalamic area (LHA) and the zona incerta (ZI), have been studied in detail. In the anterograde PHA-L staining the structure of the PHA-L stained fibers was investigated from the control rat, mesencephalic, area in the target area, projection extension. From all origins, mentioned above, fibers are distributed over the PHA-L in an intricate pattern of overlapping longitudinal columns. Newtly, most descending fibers are present in the ventral laterolateral part, including the dorsal raphe nucleus. Most caudally, some projections disappear (VMH, IHA), while others are mainly dorsolateral (PVN, DMH, LH), or the adjoining nucleus of the ventral lateral hypothalamic area (LHA). The hyperactivity usually persists for 2-3 hours and the magnitude of this phenomenon is often quite impressive. The mechanism underlying the locomotor activity may involve the non-specific release of dopamine at the target structures of damaged meninges and/or mesolimbic axons which traverse the lesion site. In order to test this hypothesis we anesthetized rats with haloperidol and observed this postoperative activity in both an open field in and photocell activity cages following injections of the dopamine antagonist haloperidol (1.0 or 2.0 mg/kg, sc). Neither dose of haloperidol blocked the activity, although at the higher dose some jumping was observed, which, however, was completely suppressed by mCPP.


On the basis of electrical brain stimulation, the hypothalamus with bicuculline methiodide elicited grooming behaviour. The role of the central and rostral PAG was studied. The central PAG is often innervated by the hypothalamus. Many of these cells are GABAergic. In order to further characterize the PR-containing GABA neurons, a retrograde tracer PHA-L was injected into the dorsal raphe nucleus. Most caudally, some projections disappear, while others are mainly dorsolateral (PVN, DMH, LH), or the adjoining nucleus of the ventral lateral hypothalamic area (LHA). The hyperactivity usually persists for 2-3 hours and the magnitude of this phenomenon is often quite impressive. The mechanism underlying the locomotor activity may involve the non-specific release of dopamine at the target structures of damaged meninges and/or mesolimbic axons which traverse the lesion site. In order to test this hypothesis we anesthetized rats with haloperidol and observed this postoperative activity in both an open field in and photocell activity cages following injections of the dopamine antagonist haloperidol (1.0 or 2.0 mg/kg, sc). Neither dose of haloperidol blocked the activity, although at the higher dose some jumping was observed, which, however, was completely suppressed by mCPP.

486.8 THE POSTOPERATIVE HYPERACTIVITY OBSERVED FOLLOWING ELECTROLYTIC LESIONS OF THE LATERAL HYPOTHALAMUS IS ATTENUATED BY MCPP, BUT NOT HALOPERIDOL. T.R. Stratford and D. Wirtshafter, Univ. Illinois at Chicago, Dept. of Psychology, Box 4348, Chicago, IL 60680

Intense locomotor activity is often observed in rats awakening from anesthesia following electrolytic lesions of the lateral hypothalamic area (LHA). The hyperactivity usually persists for 2-3 hours and the magnitude of this phenomenon is often quite impressive. The mechanism underlying the locomotor activity may involve the non-specific release of dopamine at the target structures of damaged meninges and/or mesolimbic axons which traverse the lesion site. In order to test this hypothesis we anesthetized rats with haloperidol and observed this postoperative activity in both an open field in and photocell activity cages following injections of the dopamine antagonist haloperidol (1.0 or 2.0 mg/kg, sc). Neither dose of haloperidol blocked the activity, although at the higher dose some jumping was observed, which, however, was completely suppressed by mCPP.

486.9 EFFECTS OF REPEATED ELECTRICAL STIMULATION OF THE DORSAL MEDIAL OR PARAMEDIAL NUCLEI ON GASTRODUODENAL EROSION FORMATION DURING ACTIVITY-STRESS. P.J. Geiselmann*, Dept. of Psychiatry, Psychology, UCLA, Los Angeles, CA 90024, & Dept. Psychology, Louisiana State Univ., Baton Rouge, LA 70803.

To examine the role of the centromedial amygdala (CMA) in gastric erosion formation during running activity, rats were divided into groups with or without CMA lesions and sham-operated controls were housed in Wathan activity cages. Following a 3 day baseline period (food and water ad lib), all rats were restricted to eating for 1 h each day for 21 days. Running activity, body weight, body temperature, and food intake were monitored daily. Rats with CMA lesions exhibited significantly less gastric erosions compared to sham-operated controls. There was no difference in the sham-operated controls. Those results suggest that the CMA is important in the maintenance of gastric mucosal integrity. The results also suggest that the extensive gastric damage normally viewed on ethanol feeding does not solely responsible for their eventual inanition and ultimate demise. (WIMH Fellowship 20917112, NSF BNS-8709982, and UCLA University Research Grants SPF86, P2-06)
VASOPRESSIN INNERVATION OF THE MEDIAL PREOPTIC AREA IN GERBILS ORIGINATES IN THE SUPRACHIASMATIC NUCLEUS


This study investigated the origin of vasopressin-immunoreactive (AVP-ir) fibers in the medial preoptic area (MPOA) in the gerbil hypothalamus. The MPOA contains a sexually dimorphic area (SDA) that has a subgroup of cells—the SDA pars compacta (SDApc)—which is absent in females in Nissl-stained preparations. We previously found that AVP-ir fibers were clustered in a sexually dimorphic manner in the region that houses the SDApc in males: the clusters were much larger in males than in females. We wanted to determine whether these AVP-ir fibers came from the AVP cell bodies in the bed nucleus of the stria terminalis (BST), whose efferents to the SDA are sexually dimorphic, or from the suprachiasmatic nucleus (SCN). Animals in one group received cuts dorsolateral to the SDA and ventromedial to the BST, which would transect efferents from the BST. A second group of animals received cuts ventral to the SDA and immediately dorsal to the SCN, which would transect efferents from the SCN. We found that cuts dorsal to the SCN eliminated AVP-ir fibers to the medial SDA (mSDA) and the SDAp, whereas cuts ventromedial to the BST left intact the AVP innervation of the MPOA. These results suggest that the mSDA and the SDAp receive their AVP innervation from the SCN.

SUPRACHIASMATIC NUCLEUS OF NORMAL HUMAN BRAIN: A POTENTIAL STUDY. J. Anderson and P. J. Holcomb. Dept. of Psychology, Tufts University, Medford, MA 02155.

Previous electrophysiological and behavioral studies have found similar but not identical semantic priming effects within the auditory and visual modalities. This study compared priming effects between modalities in a cross-modal lexical decision task in order to investigate the possibility of common or independent language systems for auditory and visual modalities. One group of 12 subjects was presented with visual primes and auditory targets and another 12 subjects with auditory primes and visual targets. The stimulus list consisted of 360 word pairs with equal proportions of semantically related, semantically unrelated, and word/pseudoword pairs. Three stimulus onset asynchronies (SOAs; 0, 200, and 800 msec) were randomly mixed across the experiment. When the prime was visual and the target auditory, there were large N400 effects (i.e., greater negativity for unrelated targets) at all SOAs. However, when the prime was auditory and the target visual, there were N400 effects at the 200 and 800 SOAs, but not at the 0 SOA. Similarities in the semantic priming effects in the cross-modal conditions indicate that there are common underlying priming processes in the two modalities. However, differences in the time course of the effects and in the morphology of the components indicate that semantic priming in the two modalities may not be identical. The results have implications for models of language comprehension that assume similar or different systems for the two modalities.
487.5 ELECTROPHYSIOLOGICAL EVIDENCE FOR INDIVIDUAL DIFFERENCES IN SYNTACTIC PROFICIENCY DURING SENTENCE COMPREHENSION. S.A. Koz, J. Osterhout & P.L. Holcomb, Dep. of Psychology, Tufts University, Medford, MA 02155.

Evidence from previous ERP research has shown that syntactic anomalies (e.g., verb subcategorization and phrase structure errors) elicit a positive-going component (P600) quite distinct from the N400 found for semantic anomalies. Behavioral measures were used to assess the relative stability of previously established ERP components and possible variability due to individual differences. Subjects were ranked as high and low in syntactic proficiency based on their scores from a standardized grammar test. Subsequently, event-related potentials were measured during a sentence judgement task in which subjects were required to evaluate sentences that varied in length and syntactic difficulty.

Distinct P600 effects were found for the two types of syntactic anomalies. Subcategory violations elicited a broadly distributed P600 in the low proficiency group, while the high proficiency group displayed no differences. The P600 elicited by phrase structure violations was larger at right hemisphere sites for the high proficiency group and posterior on the midline and at left hemisphere sites for the low proficiency group.

These results confirm the existence of distinct components for semantic and syntactic anomalies and indicate that the distribution and individual processing strategies of the individual speaker.


Dyslexia, or specific reading disability, may be due to anomalous development of the language areas surrounding the Sylvian fissure. Although the planum temporale is normally larger on the left, Galaburda and others have found that it is symmetrical in dyslexics.

We have examined other aspects of perisylvian morphology in two groups of dyslexics: four children with an IQ<90 discrepancy and four adults diagnosed by family history and interview. We scanned 15 age matched controls for comparison. All subjects were right handed. The adults were scanned using a volumetric acquisition that produced a gapless series of 1 mm thick sagittal images. The dyslexic children and their age matched controls were scanned with a standard T1 sequence (5 mm sagittal images). Sulcal patterns were classified with Steinmetz' method (Brain Lang. 38:515-533, 1990) as either normal (present in 82% of Steinmetz' sample and 14 out of 15 of our controls) or anomalous (extra or missing sulci).

Anomalous sulci were unexpectedly frequent in the dyslexics. Five dyslexics had extra sulci on the left while three had missing sulci on the right. Only two had normal patterns on both sides. These results support a role for anomalous brain development in the etiology of dyslexia. We conclude that thin sections in the sagittal plane afford an excellent view of the anatomical regions critical for language and that MR imaging can be used to identify behaviorally significant neuroanatomic variants.

487.8 BRAIN POTENTIALS EVOKED BY DIRECTIONAL CUES DIFFERENTIATE POOR AND GOOD READERS. L. Aniis-Vento, Dept. of Psychology, Univ. of North Carolina at Greensboro, NC 27412.

The relationship between selective spatial attention and reading ability was studied with a paradigm that included both directional and non-directional cues. The children in the study were selected in kindergarten as being at risk of developing a reading disability, and were attending the 4th grade when tested.

The task included two successive stimuli: a central cue and a peripheral target. The cue, which was either directional (a left or right arrow) or non-directional (a circle), was followed 600 ms later by a target flash appearing randomly 8° in the left or right visual field. Children were asked to respond every time the target appeared in the cued visual hemifield.

Brain potentials differed between good and poor readers starting 120 ms after arrow onset, increased over time, and were most pronounced immediately before target presentation. Good readers exhibited distinct ERP patterns in response to left and right arrows, which were greatly reduced or absent in lower readers.

Cue-related potentials, starting 320 ms after cue onset, but prior to target presentation, were correlated with reading ability, and helped explain subsequent response speed and accuracy.

Supported by NINCDS Grant ROI NS19413-07

487.9 PRESERVED ACCESS AND PROCESSING OF SOCIAL KNOWLEDGE IN ACQUIRED SOCIOPATHY CAUSED BY VENTROMEDIAL FRONTAL DAMAGE. J. Saver*, A.R. Damasio, D. Trancl. Div. of Behav. Neurology & Cognitive Neuroscience, U. Iowa College of Medicine, Iowa City, IA 52242.

Dyslexia, or specific reading disability, may be due to anomalous development of the language areas surrounding the Sylvian fissure. Although the planum temporale is normally larger on the left, Galaburda and others have found that it is symmetrical in dyslexics.

We have examined other aspects of perisylvian morphology in two groups of dyslexics: four children with an IQ<90 discrepancy and four adults diagnosed by family history and interview. We scanned 15 age matched controls for comparison. All subjects were right handed. The adults were scanned using a volumetric acquisition that produced a gapless series of 1 mm thick sagittal images. The dyslexic children and their age matched controls were scanned with a standard T1 sequence (5 mm sagittal images). Sulcal patterns were classified with Steinmetz' method (Brain Lang. 38:515-533, 1990) as either normal (present in 82% of Steinmetz' sample and 14 out of 15 of our controls) or anomalous (extra or missing sulci).

Anomalous sulci were unexpectedly frequent in the dyslexics. Five dyslexics had extra sulci on the left while three had missing sulci on the right. Only two had normal patterns on both sides. These results support a role for anomalous brain development in the etiology of dyslexia. We conclude that thin sections in the sagittal plane afford an excellent view of the anatomical regions critical for language and that MR imaging can be used to identify behaviorally significant neuroanatomic variants.

487.10 LAUGHTER: SONIC STRUCTURE AND CONTAGION. R. R. Provine, Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

Laughter is an ancient mode of pre-linguistic vocal communication performed in parallel with modern speech. A Sonographic analysis showed laughter is characterized by stereotyped laugh-note duration ("ha", X = 75 ms) and internote interval, a laughter specific acoustic feature detector. Although the existence of laughter is well-known and the basis of "laugh tracks" on broadcast comedy shows, the implications of this potent and extraordinary behavior for brain function are unappreciated.

It is proposed here that stereotypical laughter is produced by a motor pattern generator that, in the case of contagious laughter, is triggered by a laugh-specific acoustic feature detector. Although the existence of human auditory feature detectors is debated, detectors are more likely for simple, species-typical laughter than for complex and culturally variable speech. Laughter and its contagion have numerous implications for theories of speech perception, the evolution of speech and language, the neural basis of social behavior, and cognitive neuroscience.
Disruptions in CNS catecholamine systems have been postulated to underlie cognitive deficits in patients with a variety of conditions, including subcortical dementia, schizophrenia, and major depression. The effect of catecholamine depletion on cognitive performance in 40 healthy adult males was tested by preventing synthesis of catecholamines with AMPT, an inhibitor of tyrosine hydroxylase. Subjects were randomized to sleep deprived (no sleep for 40.5 hrs) or rested (7 1/2 hrs/night) conditions. In both sleep conditions, subjects received oral AMPT (or lactose placebo) at a dose of 750 mg QID over two days, for a total of 7 doses. Cognitive performance was measured repeatedly during the two days of drug administration and the day following the last drug dose, using a computerized battery of seven cognitive tasks. AMPT treatment alone, like sleep deprivation alone, was associated with decrements in cognitive performance. Furthermore, the combination of sleep deprivation and AMPT treatment was associated with greater performance decrements than either treatment alone. These findings support the view that catecholamines play a role in normal cognitive processes.

Further, they suggest that decrements in cognitive performance associated with sleep deprivation may be mediated by the catecholamine neurotransmitters. Sleep deprivation may be a useful tool for detecting subclinical cognitive dysfunction in patients with a suspected cognitive deficit.

**487.13**

**MOTOR DYSFUNCTION IN EARLY ASYMPTOMATIC HIV INFECTION.** B.A. Stern,* N.G. Singer,* S.G. Silva,* H.J. Rogers,* D.O. Perkins,* C.D. Hall,* C.M. van der Heul,* D.E. Evans, Dept. of Neurology, National Institutes of Health, Bethesda, MD, 20892, U.S.A.

AIDS frequently results in CNS dysfunction. It remains unclear, however, whether asymptomatic HIV seropositive individuals exhibit neurobehavioral deficits early in the course of infection, prior to the development of symptoms and signs. The goal of this study was to assess the impact of potential co-factors on the early presentation of cognitive and/or motor disturbance in HIV. Subjects included 35 asymptomatic HIV seropositive gay men and 44 seronegative gay male controls. Strict exclusion criteria were used to ensure that subjects were healthy. Neuropsychological evaluations were conducted as part of a larger, longitudinal study. Group differences in summary ratings and individual test scores were first assessed with analyses of variance. Education, SES, B12 and folate, and mood-state, which was assessed using several standardized tests, such as the Profile of Mood States, were used to control for confounding variables. Significant group differences were found in a variety of areas, with the seropositive subjects performing worse than controls. For example, on the Rey-Osterreith Complex Figure test, the seropositive subjects performed significantly worse than the controls. These significant group differences remained after controlling for SES, CD4 count, B12 level, and mood. In addition, there were no significant differences in adverse experiences, urinary organic acids, and electroencephalograms when HIV was compared to placebo. These results reaffirm the safety of APM in PKUH and refute the speculation that APM affects cognitive performance.

**487.14**

**ABSENCE OF NEUROBEHAVIORAL EFFECTS OF HIGH DOSE ASPARTAME IN PHENYLETHERONIC HETEROREZYGOTE (PKUH).** P. R. Solomon, M.E. Gocmen-Altin, K.R. Edwards, and M.E. Stanton, Department of Neurology, New York University School of Medicine, New York City, New York, 10016

A common problem in psychopharmacology and neuropsychology is the absence of a reliable method to compare the sensitivity of behavioral tasks employed in humans. Most environmental, behavioral, and psychopharmacologic manipulations do not consistently produce readily detectable, graded deficits in human performance and mood. However, human cognitive function is sensitive to changes in oxygen availability and exposure to hypoxia should produce a continuum of effects at altitude level and duration increased. We evaluated the behavioral effects of hypoxia as a function of time of exposure and altitude level with various standardized tests of cognitive function and mood-state. Twenty-three males were tested in an altitude chamber during a 4.5 hour exposure to hypobaric hypoxia. All subjects were exposed to two levels of hypoxia (4200 m and 4700 m) and a near sea level (550 m) control condition. Prior to altitude exposure, extensive practice was given on all tests. Cognitive function was significantly impaired on most tests. Even on relatively simple tasks, such as simple and choice reaction time, as well as complex tests of cognition, such as the Tower of Hanoi, function was significantly reduced in a graded manner. Mood-state, which was assessed using several standardized tests, such as the Profile of Mood States, was also impaired in a graded manner. In general, the magnitude of the effects observed was a function of exposure duration and altitude level. The robust and consistent effects observed and their graded, relatively non-specific nature, suggests that graded exposure to hypobaric hypoxia may be a useful tool for comparing the sensitivity of various tests of human performance, cognition and mood.
488.3

LACK OF AMNESIC EFFECT OF SCOPOLAMINE IN UNDER-

Experimental data indicate that in conditions of overtraining or over-
reinforcement cholinergic activity is not needed for memory consolidation. The aim of this work was to determine if underreinforcement and over-
reinforcement conditions are important factors in cholinergic activity that is not needed for memory consolidation. We trained rats to perform a task of passive avoidance and we gave one footshock (under-
reinforcement, 4 and 8 V) or two footshocks (overreinforcement) to one of the animals. The other half of the animals was trained with a true baseline (no shock). True baseline consolidation of passive avoidance, trained with low levels of footshock (under-
reinforcement, 4 V) was not affected to one footshock during training (0.0, 0.3, 0.4, 0.5, 0.6, 0.7, or 0.8 mA). Half the groups was given one ip injection of scopolamine (8 mg/kg), 5 min after training and the other half was not injected. Scopolamine-induced amnesia was found only in the animals trained with 0.7 and 0.8 mA. These data suggest that muscarinic cholinergic activity is not involved in memory consolidation of under-reinforced tasks.

Supported by CONACYT (D11-903541) and DGAPA, UNAM.

488.8


Rats were trained in an operant spatial delayed alternation task employing various delays ranging from 5 to 240 s. In addition, performance of the levers during retention intervals were recorded and analyzed. In untreated animals, the relative number of correct responses and correct lever responses (operations) was determined for each choice over the following correct response (operations) varied with the length of the retention intervals, and these measures were correlated. The response rate for lever responses increased with the length of the delay (which was correlated with the underlying tendency to alternate between lever types). This effect may have contributed to the delay-dependency of correct responding.

Scopolamine-hydrobromide (0.01, 0.03, 0.1, 0.3 mg/kg) and -methylbromide (0.1, 0.3 mg/kg) produced main effects on correct responding, but did not interfere with the relative number of correct lever responses. As both scopolamine-derived effects did not affect the relative number of correct lever responses but of correct responses, it is speculated that these treatments increased the probability that the onset of the panel light (that was assumed to indicate lever activation) triggered a repositioning response, yielding a delay-independent reduction in the number of correct responses. These results suggest that in tasks tasks that allow the development of specific ordering of lever responses, delay-dependent response accuracy does not represent a sufficient condition for conclusions on task demands on memory. Furthermore, it seems that drug-induced changes in such tasks may be a result of alterations within peripheral functions.

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THURSDAY AM

Brain Research Institute. Department of Pharmacology and Experimental Therapeutics, The Scripps Research Institute, La Jolla, CA 92037.

Somatic analogues of soman (dimethyl-Pro-THP) are known to reverse an atropine-induced water maze deficit. We evaluated whether RX77368 could attenuate a scopolamine-induced deficit in the radial arm maze. Rats were cannulated and subjected to a 23-hr deprivation schedule then tested on 12-16, 19-23 and 26-30 post-surgery in a radial maze. Four groups of rats (n=8 each) were pre-trained with either saline (SAL) or RX77368 (3 mg/kg) followed 10 min later with either SAL or RX77368 (3 mg/kg). Rats were trained on a radial maze with a goal arm discrimination in a T-maze. In addition, the RX77368-treated mice showed improved recent recall one week following initial discrimination learning. Physostigmine and hydroygerine also facilitated retention of discriminated escape in the young mice. Pre-treatment with RX77368 (from 0.64 to 10 mg/kg) was effective in reducing or eliminating amnesia induced by treatment with the anticholinergic, scopolamine. Hydroygerine was less effective than RX77368 in the drug-induced amnesia paradigm. In the third model, daily injections of RX77368 prior to testing improved acquisition of an active avoidance response in 4-month-old NZB/BINJ mice, a genotype noted for accelerated age-related decline of learning capacity (Forster & Lal, Br. Res. Bull, 25:503-516, 1990). These results suggest that RX77368 may be effective in improving cognitive ability in both normal subjects and individuals with age-related memory impairment. (Supported by a research grant from Bristol Myers Squibb Company and NIH grant AG06182).


Butyrylcholinesterase (BuChE) can prevent death and signs of nerve agent intoxication. SPR performance was significantly improved over time, as measured by untrained arm entries (UE, P<0.05), total escape latency (TSL; P<0.05) and total maze time (P<0.01). UE were significantly reduced by SCP alone (P<0.05; days 13-29), indicating an SCP-induced learning deficit. SCP alone also significantly improved rear entries (RE) at the start of the trials (P<0.05) and significantly more at the end (P<0.05), suggesting SCP had non-cognitive effects. RX77368 significantly attenuated the SCP-induced deficit on day 13-21 but not on later days. RX77368 alone significantly increased UE (P<0.05, days 12, 15) compared to SAL, suggesting that the TRH analogue either alone or in combination with SCP improved performance. Hypothalamic TRH levels (SAL; 169±18 pg/mg protein) were significantly elevated in the SAL/SCP (580.5±106.6, P<0.01), RX77368/SAL (378.5±75.4, P<0.05) and RX77368/SCP (534/49, P<0.05) groups. Changes in other areas were less pronounced. Taken together these results suggest that SCP-induced radial maze performance deficit may result from a reduction in locomotion rather than solely a learning impairment and that RX77368 may improve this impaired end of the task.

C.D.W. is a SERC CASE Student with Reckitt & Colman.


Reversible inhibitors of ACHE (E2020, GEEF, Tacrine) as compared with irreversible inhibitors (DFP, VX2150, SA-1250) were studied in the plasma and red blood cells of humans (55 males; mean age 36 yrs). Structures of E2020 are similar to those of insecticides. In vitro, E2020 binds irreversibly with ACHE but has normal kinetic properties. The effectiveness of E2020 in humans was assessed by measuring plasma and red blood cell ACHE activity and determining the efficacy of E2020 in preventing DFP antagonism in red blood cells (RBC) from patients treated with E2020. The activity of ACHE in plasma and red blood cells was measured at 262.5-fold dilution to prevent further action of DFP and to fully dissociate DFP. A single oral dose of E2020 (2 mg/kg; s.c) produced marked and sustained DFP antagonism in rat cortex (>30% for >50% of 5 hr) and in human plasma. The ability to protect ACHE against irreversible inhibition by DFP in vitro thus provides a sensitive, new approach for quantifying the pharmacodynamics of these reversible inhibitors. In the present studies, we show that plasma and RBC ACHE in red blood cells and plasma are antagonized closely correlated to log concentration of E2020 in plasma. With chronic dosing, plasma and red blood cell ACHE activity increased significantly reduced by SCP alone (P<0.01, days 13-29), indicating a SCP-induced deficit on days 13-21 but not on later days. RX77368 alone significantly increased UE (P<0.05, days 12, 15) compared to SAL, suggesting that the TRH analogue either alone or in combination with SCP improved performance. Hypothalamic TRH levels (SAL; 169±18 pg/mg protein) were significantly elevated in the SAL/SCP (580.5±106.6, P<0.01), RX77368/SAL (378.5±75.4, P<0.05) and RX77368/SCP (534/49, P<0.05) groups. Changes in other areas were less pronounced. Taken together these results suggest that SCP-induced radial maze performance deficit may result from a reduction in locomotion rather than solely a learning impairment and that RX77368 may improve this impaired end of the task.

C.D.W. is a SERC CASE Student with Reckitt & Colman.

BMY 21502: A COMPARISON WITH HYDROGERINE® AND PHYSOSTIGMINE. H. Lal, K.C. Heitz, and M.J. Forster, Dept. of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107. MBD 71502, a substituted pyridomide, was tested in three animal models for its potential to reverse age-related memory dysfunction. In two of the models, efficacy of BMY 21502 was compared with physostigmine and hydroygerine®. BMY 21502 treatment prior to and continued during testing improved performance in a goal arm discrimination in a T-maze. In addition, the BMY 21502-treated mice showed improved recent recall one week following initial discrimination learning. Physostigmine and hydroygerine® also facilitated retention of discriminated escape in the young mice. Pre-treatment with BMY 21502 (from 0.64 to 10 mg/kg) was effective in reducing or eliminating amnesia induced by treatment with the anticholinergic, scopolamine. Hydroygerine was less effective than BMY 21502 in the drug-induced amnesia paradigm. In the third model, daily injections of BMY 21502 prior to testing improved acquisition of an active avoidance response in 4-month-old NZB/BINJ mice, a genotype noted for accelerated age-related decline of learning capacity (Forster & Lal, Br. Res. Bull, 25:503-516, 1990). These results suggest that BMY 21502 may be effective in improving cognitive ability in both normal subjects and individuals with age-related memory impairment. (Supported by a research grant from Bristol Myers Squibb Company and NIH grant AG06182).


Reversible inhibitors of ACHE (E2020, GEEF, Tacrine) as compared with irreversible inhibitors (DFP, VX2150, SA-1250) were studied in the plasma and red blood cells of humans (55 males; mean age 36 yrs). Structures of E2020 are similar to those of insecticides. In vitro, E2020 binds irreversibly with ACHE but has normal kinetic properties. The effectiveness of E2020 in humans was assessed by measuring plasma and red blood cell ACHE activity and determining the efficacy of E2020 in preventing DFP antagonism in red blood cells (RBC) from patients treated with E2020. The activity of ACHE in plasma and red blood cells was measured at 262.5-fold dilution to prevent further action of DFP and to fully dissociate DFP. A single oral dose of E2020 (2 mg/kg; s.c) produced marked and sustained DFP antagonism in rat cortex (>30% for >50% of 5 hr) and in human plasma. The ability to protect ACHE against irreversible inhibition by DFP in vitro thus provides a sensitive, new approach for quantifying the pharmacodynamics of these reversible inhibitors. In the present studies, we show that plasma and RBC ACHE in red blood cells and plasma are antagonized closely correlated to log concentration of E2020 in plasma. With chronic dosing, plasma and red blood cell ACHE activity increased significantly reduced by SCP alone (P<0.01, days 13-29), indicating a SCP-induced deficit on days 13-21 but not on later days. RX77368 alone significantly increased UE (P<0.05, days 12, 15) compared to SAL, suggesting that the TRH analogue either alone or in combination with SCP improved performance. Hypothalamic TRH levels (SAL; 169±18 pg/mg protein) were significantly elevated in the SAL/SCP (580.5±106.6, P<0.01), RX77368/SAL (378.5±75.4, P<0.05) and RX77368/SCP (534/49, P<0.05) groups. Changes in other areas were less pronounced. Taken together these results suggest that SCP-induced radial maze performance deficit may result from a reduction in locomotion rather than solely a learning impairment and that RX77368 may improve this impaired end of the task.

C.D.W. is a SERC CASE Student with Reckitt & Colman.

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488.15

Nicotinic agents have been implicated as modulators of learning and memory. Agonists tend to disrupt learning and memory. This study compared the ability of mecamylamine (MEC) and chlorisondamine (CHLOR), two central nicotinic antagonists, to disrupt nicotine's (NIC) agonist effects on locomotion and learning. Pretraining administration of NIC (0.03 mg/kg max effect) enhanced 24 hour retention of an inhibitory avoidance task. Chronic CHLOR pretreatment (10 μg, icv), did not antagonize the enhanced retention produced by NIC. Premedication of the peripheral antagonist MEC (1.0 mg/kg), did effectively block the enhancement produced by pretraining NIC. Premedication of the peripheral nicotinic antagonist hexamethonium (5.0 mg/kg), was not able to block the memory enhancing effect of nicotine. Animals from the previous experiments were then challenged with an acute dose of NIC (0.01, 0.1, 0.3 mg/kg, ip) and placed in activity monitors for 60 min. CHLOR blocked both the depressant and stimulatory effects of NIC. In a separate group of animals MEC (1.0 mg/kg, ip) was administered 16 minutes prior to the challenge with NIC. MEC produced a comparable blockade of NIC. CONCLUSION: CHLOR appears to be a very potent antagonist of NIC with respect to locomotor activity, yet does not disrupt the enhancement of cognition by NIC. However, MEC is able to disrupt cognition as doses that are as effective as CHLOR in blocking NIC's locomotor effect. Therefore, the ability of a nicotinic antagonist to block the locomotor effects of an agonist does not appear to predict its ability to disrupt the effects of an agonist in a cognitive task. The present findings suggest that the nicotinic agonists may act in different sizes, and could be useful tools in differentiating nicotinic receptor subtypes.

488.17
NICOTINE-INDUCED FACILITATION OF RADIAL-ARM MAZE PERFORMANCE IN RATS. E.D. Levin, F.H. Brucato*, H.S. Swartzwelder and J.E. Rose. Departments of Psychiatry and Neurology, University of Maryland School of Medicine, Baltimore, MD 21201.

Abundant research has demonstrated the importance of muscarinic acetylcholine (ACh) receptors for cognitive function. Nicotinic ACh receptors have received less attention, but they are also appear to be important for cognitive function. We have found that like muscarinic blockade nicotinic blockade impairs choice accuracy in the radial-arm maze. Facilitation of nicotinic receptors with nicotine improves radial-arm maze choice accuracy after either acute (1.0 μg/kg) or chronic (subcutaneous injection of 0.6 μg/kg/day for 3 days) nicotine treatment. In a radial-arm maze exploration paradigm female Sprague-Dawley rats (N=13) averaged 3.95±0.48 (mean±sem) entries to repeat after control infusions and 4.87±0.31 after nicotine infusions (p<0.05). The dose of the nicotinic antagonist mecamylamine tried thus far (200 μg) did not significantly alter choice accuracy. Alzheimer's patients show decreased cortical nicotinic receptor binding. Additional nicotinic stimulation may help to reverse their cognitive impairment. (Supported by an Alzheimer's Association/Neil Bluhm Research Grant and NIDA grant 20665)

488.18
EFFECTS OF CHRONIC NICOTINE ADMINISTRATION ON THE COGNITIVE PERFORMANCE OF AGED RATS. G.W. Arendash and G.J. Seongock. Department of Biology and Institute for Biomolecular Science, University of South Florida, Tampa, Florida 33620.

Aged rats have been shown to be impaired in the acquisition and/or retention of a wide variety of cognitive tasks. Despite reports showing that nicotine administration to young adult rats can affect cognitive performance, the effects of nicotine in aged rats have not yet been investigated. The purpose of this study was, firstly, to characterize the effects of aging on three dissimilar tasks and, secondly, to elucidate the effects of daily nicotine administration on the performance of aged rats in these tasks. To accomplish this, male Sprague-Dawley rats aged 2-3 months (young) and 22-24 months (aged) were tested in one of the following tasks: 14-arm radial maze, one-way active avoidance, or Lakesh Island maze performance. Aged rats received a p.i. injection of nicotine (0.2 mg/kg) or saline vehicle 15 minutes prior to daily behavioral testing; young control rats received saline vehicle injections. Cognitive performance deficits observed in aged rats were significantly impaired in the general learning and reference (long-term) memory components of 14-arm maze performance; working (short-term) memory was not affected. In contrast, nicotine pretreatment improved acquisition in both general learning and reference memory of aged rats in this task. An age-related difference in the acquisition of one-way active avoidance pole jumping was also found. Nicotine pretreatment of similar aged rats was able to markedly improve overall learning and rate of learning compared to aged controls. In Lakesh Island maze testing, aged control rats made substantially more errors than young control rats; aged rats pretreated with nicotine made less errors than aged controls. These results indicate that chronic nicotine administration can improve the impaired learning/memory abilities of aged rats in several different tasks.

488.19
MEMORY IN THE MONKEY: AN AUTOMATED PARADIGM AND THE EFFECT OF NICOTINE. J.W. Ashford and H. Edwards*. Department of Psychiatry, University of California, Davis. VA Medical Center, Martinez, CA, 94553.

This study examined the formation of memory which is not disrupted by interference. The study used rhesus monkeys in a new automated testing paradigm. Monkeys learned to push a joystick lever to the left or the right in response to a video screen letter stimulus (7cm x 5cm) for a juice reward. Four animals learned to discriminate 12 letters: A B C D E F G H I J K L, learned to push a joystick lever to the left or the right in response to a video screen letter stimulus (7cm x 5cm) for a juice reward. Four animals learned to discriminate 12 letters: A B C D E F G H I J K L. Letters were introduced in directionally opposed pairs, 10 consecutive correct responses for each direction pair were required to proceed to the learning of the next pair. If the animal learned all of the pairs in less than an hour, he was tested for the remainder of the hour on the sequence E F G H I J K L. One animal could learn the associations within 30 min, then score 99% correctly during the remaining 30 min (J.L. cog. int. triails/320 total). To test the effect of nicotine in this paradigm, doses up to 100μg/kg i.m. were administered 5 minutes before the task began. The animal was then placed in a dark, dimly lit room and the task performance was observed. In all four animals: the optimal dose for 1 animal was 2.5μg/kg, and for the other 3 was 5μg/kg. Such automated tasks will facilitate analysis of memory in primates. (Supported by a donation from R.J. Reynolds Tobacco Co.)

488.20
NICOTINE REPLACEMENT AFTER 12-HR ABSTINENCE REDUCED MIRROR-TRACING TIME BUT DIFFERENTIALLY AFFECTED SUPRASPIN DIGIT LEARNING IN TYPE A AND B SUBJECTS. R.-Z. Rao, S. Armick and D. Moss. Dep't. of Psychol., Wabash College, Crawfordsville, IN 47933.

A supraspin (18) digit learning (SDL) and a mirror-tracing (MT) task were studied with five repetitions each. Computerized visual signal detection and Type A and B personality (TA & TB) were also assessed. Male college student smokers (N=18) and chewers (N=16) were randomly assigned to one of three treatment conditions after 12-hr abstinence: no replacement, low dose, and high dose (1/2, and 1) cigarette, or nicotine gum (2, and 4 mg), respectively. The two replacement groups were combined, as were the smokers and chewers, for analyses. Nicotine replacement had no effect on signal detection but reduced MT time during the initial trials without affecting MT error; nicotine also enhanced supraspin digit learning in TA subjects (p<0.05, t=3.45) but impaired it in TB subjects (p<0.05, t=-2.81). The results suggest that changes in cognitive effects of nicotine may be used to account for their psychological factors associated with coronary heart disease risks.
489.1 
DORSOMEDIAL HYPOTHALAMIC LESIONS FAIL TO DISRUPT SELF-STIMULATION OF THE CAUDAL MFB. 
It has been proposed that the dorsomedial hypothalamic nucleus (DMH) gives rise to most or all of the substrate for the rewarding effect of stimulating the medial forebrain bundle (MFB) (Glimcher and Gallistel, Soc. Neurosci. Abstr., 1989, 15, 33). To test this hypothesis, a caudal MFB stimulation electrode and an ipsilateral DMH lesioning electrode were implanted in male Long Evans rats. Rate-frequency curves were collected at each of 2-3 stimulation currents. For some subjects, the minimum effective stimulation current was also determined. 
Neither measure of rewarding effectiveness was altered by DMH damage in 6 rats. Some decrease in rewarding effectiveness was noted in the case of two rats in which the lesions extended beyond the DMH to invade the perifornical lateral hypothalmus and caudal MFB. These results challenge the notion that the DMH gives rise to all or most of the substrate for MFB reward. Nonetheless, they do not rule out the possibility that reward fibers in some part of the caudal MFB arise in the DMH.

489.2 
ATTENUATION OF MFB REWARD BY ROSTRAL LH LESIONS DEPENDS ON STIMULATION SITE AND CURRENT. B. Murray and P. Shizgal. Concordia Univ., Montreal, Quebec H3G 1M8. 
We have previously reported that electrolytic lesions of the antero-lateral portion of the lateral hypothalamus (LH) can reduce the rewarding impact of electrically stimulating more posterior MFB sites (Soc. Neurosci. Abstr., 1988, 14, 1101); in one subject, the effect of the lesion varied with the stimulation current. To further investigate these phenomena, electrodes were implanted in male rats at 3 levels of the MFB: the anterior LH, middle LH, and anterior ventral tegmental area (VTA). Rate-frequency curves were collected using 3 currents at each middle LH and VTA site. 
Electrolytic lesions (anodal, 1.0 mA for 10 sec) were made through the anterior LH electrode. In 4 subjects, lesions of the anterior LH produced long-lasting increases of up to 78% in the frequency threshold for self-stimulation of the middle LH and/or VTA sites. The magnitude of these effects was highly dependent upon the stimulation site and current. The lesions failed to alter the frequency threshold in 5 other subjects. These data suggest a precise spatial relationship between reward-relevant neural elements in the anterior, middle, and posterior MFB.

489.3 
THE EFFECT OF PERIPHERALLY ADMINISTERED BOMBESIN ON LH STIMULATION-INDUCED FEEDING AND SELF-STIMULATION. T. Harris, C. Bielajew, and L. Marketing School of Psychology, Univ. of Ottawa, Ottawa, Ontario, Canada. 
Bombesin's purported role in satiety mechanisms prompted this investigation of the effect of peripheral hypothalamic (IH) stimulation-induced feeding (SIF) and self-stimulation (SS); these behaviors can be elicited from the same site and may share some common neuronal elements. Using a Latin Square design, SIF and SS thresholds derived from the same electrode were assessed in 8 rats following four doses of bombesin: 0, 2, 4, and 8 μg/kg i.p. An additional dose of 16 μg/kg was tested in three of the animals. In SIF trials, a 20 sec or 50 sec or off alternating cycle of pulse periods was delivered on an ascending logarithmic scale; the threshold was defined as the first period that failed to elicit feeding. SS period thresholds were interpolated from descending rate/pulse functions using a constant response criterion. For each behavior, thresholds were collected at six current values, ranging from 80 μA to 800 μA. Bombesin at any dose had no effect on SS thresholds but slightly lowered SIF period thresholds at the higher concentrations. These results suggest that, unlike other satiety factors (e.g., CCK), peripheral bombesin mechanisms are less involved in SS-induced reward and feeding.

Supported by NSERC Grant #05541 to C.B.

489.4 
MORPHINE MICROINJECTION INTO THE VENTRAL SEGMENTAL AREA (VTA): EFFECTS OF REPEATED ADMINISTRATION ON LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD. P. Baou, Y. Wans* and R.A. Wise. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G 1M8. 
While some of the behavioral properties of opiates show tolerance following repeated administration, the locomotor effects of morphine injections into the VTA show sensitization or "reverse-tolerance." VTA morphine also facilitates the rewarding effects of lateral hypothalamic electrical stimulation; we assessed the effects of repeated injections to determine whether there is sensitization of this opiate action. Unilateral injections of VTA morphine (2.5μg/0.5μl) were given on alternate days for a total of 10 treatments. These treatments caused leftward shifts in the function relating response rate to stimulation frequency, lowering the "dose" of stimulation required to maintain responding at the pre-treatment level but without altering the maximum rates of responding seen with high stimulation doses. There was no apparent sensitization of the ability of morphine to potentiate the rewarding effects of brain stimulation reward. If anything there was a decrease in effectiveness over the course of treatments. This decrease in effectiveness may reflect some degree of pharmacological tolerance or it may reflect local tissue changes caused by the repeated central injections.

489.5 
The medial forebrain bundle (MFB) at the level of the lateral hypothalamus (LH) is probably most commonly studied in brain-stimulation reward (BSR) experiments. Although the MFB is rich in dopaminergic (DA), cholinergic (ACH), adrenergic, and serotonergic (5-HT) fibers of passage, current work has mainly implicated the DA system for the rewarding effects of BSR, with ACH and 5-HT serving a complementary inhibitory role. Among the sources of 5-HT fibers in the MFB is the ventral tegmental nucleus of Gudden (VtG) which gives rise to ascending fibers to the median raphe nucleus, VTA, MFB and other structures. The majority of studies suggest an inhibitory role for 5-HT in self-stimulation behavior used the MFB-LH as the stimulation site. To investigate the possible role of direct stimulation of 5-HT in BSR, bipolar electrodes were implanted in the VtG and the MFB-LH, respectively, in each of four male P-3H4 rats. BSR thresholds were determined at each site. The results suggest that VtG stimulation is as effective as MFB stimulation in learning rate maintenance of reinforcing behavior. We conclude that VtG is a novel reinforcing site suggesting that 5-HT may have rewarding properties (Supported in part by NIDA grant DA02526 and Research Scientist Award DA00999 to CK).

489.6 
THE EFFECTS OF REPEATED PHENCYCLIDINE (PCP) ON LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD (BSR) AND LOCOMOTION IN RATS. W.A. Carlson, JR. and R.A. Wise. Center for Studies in Behavioral Neurobiology. Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8. 
It has been suggested that the rewarding effects of psychomotor stimulants are intimately associated with forward locomotion in rats. Phencyclidine (PCP), like most drugs that are rewarding in their own right, potentiates lateral hypothalamic brain stimulation reward (BSR). In rats trained to lever press for electrical stimulation of the medial forebrain bundle (MFB), this facilitation was demonstrated after acute systemic administration of PCP (2.5 and 5.0 mg/kg) by leftward shifts in the functions that related response rate to stimulation frequency; that is, a lower than normal level of stimulation was sufficient to maintain baseline responding in phencyclidine-treated animals. When animals were tested in the BSR paradigm once per week for 8 weeks, no progressive increases in the facilitation effects of these doses of PCP were observed. On the other hand, another group of animals administered PCP (5.0 mg/kg) in a chamber outfitted with photocells demonstrated a progressive increase in sensitivity to the locomotor-stimulating effects of the drug with repeated testing. Thus, these data suggest that the reward-facilitating and at least one of the psychomotor stimulant effects of this drug can be dissociated.
FAILURE OF CHRONIC ESTROGEN TO ALTER LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD. C.M Podlak & M.A. Bozarth, Department of Psychology, University at Buffalo, Buffalo, NY 14260.

The threshold tracking procedure was used to determine the effect of chronic estrogen replacement after ovariectomy on brain stimulation reward (BSR). Female, Long-Evans rats were implanted with monopolar electrodes aimed at the lateral hypothalamic level of the medial forebrain bundle. After 5 to 10 days recovery from surgery, rats were trained to lever press for monophasic cathodal stimulation pulses during 60-minute daily test sessions. The threshold tracking procedure determined the minimum stimulation frequency that maintains a predetermined response rate (i.e., 30 presses/min). Following ovariectomy, 20 μg of 17β-Estradiol was subcutaneously injected for 4 days followed by 4 days of vehicle injections; this injection series was repeated for 3 cycles. Chronically administered estradiol had no effect on BSR thresholds. These data along with previous data examining the effect of acute estrogen (Podlak & Bozarth, Soc. Neurosci. Abstr., 1990) suggest that estrogen does not influence the rewarding impact of lateral hypothalamic BSR.

MODELLING DRUG SELF-ADMINISTRATION WITH SELF-ADMINISTRATION OF BRAIN STIMULATION (SABS): STABILITY OF SABS WITH PARTIAL REINFORCEMENT SCHEDULES. Mario Lepecq*, Keith B.J. Franklin and Frances V. Abbott., McGill University, 1205 Dr Penfield Ave, Montreal, Que., Canada H3A 1B1

We have previously shown that rats trained on conventional self-stimulation will self-administer trains of brain stimulation that rise and fall in pulse frequency with a time course that mimics the rise and fall of drug concentrations in the brain. We now report that rats will spontaneously acquire such self-administration of brain stimulation, and that the pattern of behaviour is stable when "doses" of brain stimulation are delivered on a partial reinforcement schedule. As response requirement is increased from FR3 to FR15, the mean response rate increases while the mean frequency of stimulation (analogous to mean plasma drug concentration) decreases. This relationship is also found in drug self-administration. Another characteristic feature of drug self-administration is the rate of self-administration decreases as the elimination half-life of the drug increases. This characteristic is also mimicked by SABS on a partial reinforcement schedule. Using single frequency modulated 16 sec trains of brain stimulation as reinforcers on an F160 sec schedule we have shown that the rate of "absorption" contributes to the overall reinforcing effect of a stimulation train.


Stimulus rate differences have been observed between Intracranial Electrical Self-Stimulation (ICSS) and Intravenous Drug Self-Administration in rats. Recently these differences have been attributed to temporal parameters (Franklin et al, NS Abstr:15:1092, 1989). In our study, ICSS was delivered in a "cocaine-like" waveform in which pulse frequency changed each second over a 50-150 second period, rising quickly to a peak and then decaying slowly. Responding during a waveform triggered a new waveform which was added to the ongoing one. As with cocaine, rats responded with periods of high lever press rates alternating with periods of abstinence. Decreasing ICSS current, or "dose", resulted in increased responding with some rats following the standard ICSS psychophysical trade-off between current and self-selected pulse frequency. Decreasing the duration of the waveform also resulted in increased responding. (Supported by the Whitehall Foundation)

EFFECTS OF LATERAL PREOPTIC AREA KNIFE-CUTS ON POSTERIOR HYPOTHALAMIC SELF-STIMULATION REWARD J.R. Stellar and Hugh Albert*, Psychology Dept., Northeastern University, Boston, MA 02115.

Previously we reported that coronal knife-cuts placed in mid-lateral hypothalamus could be effective in reducing posterior hypothalamic self-stimulation reward in the rate-frequency paradigm using multiple stimulus currents. This effect was particularly strong at low currents (Stellar et al, NS Abstr:16:591, 1990). This year we extended these findings to coronal knife-cuts placed farther anterior, in the lateral preoptic area, keeping electrode placement the same. Half-maximal rate-frequency reward thresholds were raised (reward decrease) about 0.1 - 0.4 log Hz and were related to the size of the knife cut. Cuts ranged from minimal ventral damage just above the optic chiasm to a medial forebrain bundle transection that ran the entire dorsal-ventral length of the lateral preoptic area. Reward thresholds were less dependent on current than previously reported, suggesting that electrode/knife-cut alignment was not as important with these placements. (Supported by the Whitehall Foundation)

EFFECTS OF MOTIVATIONAL STATE ON OPERANT BEHAVIORS DIFFERENTIALLY SENSITIVE TO CHANGES IN REINFORCER VALUE. M. Vigorito, J.C. Carretta*, C.B. Kruse* and S.A. Radice*, Dept. of Psychology, Seton Hall University, South Orange, N J 07079.

Rate-reducing effects of neuroleptic drugs on operant behaviors reinforced with food have been interpreted as resulting from the reduction in the reward value of the reinforcer. However, operant response rates are not always sensitive to changes in the value of the reinforcer. We previously found that in well-trained hungry rats VR rates were more sensitive to reductions in the value of a sucrose reward than VI rates; increases in the value of the reinforcer did not elevate response rates in either schedule. In the present experiment we replicated our earlier findings when rats were hungry and found that when the rats were not hungry sensitivity to reinforcer reductions was enhanced in both schedules. Moreover, an increase in the reinforcer value under the ad libitum condition elevated VI rates but not VR rates. We suggest that the pharmacological manipulation of operant behaviors that are differentially sensitive to changes in reinforcer value can be useful in understanding how drugs alter behaviors motivated by food reward.

LOCUS COERULEUS STIMULATION-INDUCED PLACE PREFERENCES ARE PREVENTED BY PRETREATMENT WITH THE DOPAMINE ANTAGONIST PIMZIDE. C.L. Duvauchelle, L.A. MacConell*, A. Eremia and A. Eitenberg, Behavioral Pharmacology Lab, Dept. of Psychology, University of California, Santa Barbara, CA 93106.

Electrical stimulation of the norepinephrine cell bodies in the locus coeruleus (LC) has been shown to support self-stimulation behaviors in rats. The present study employed a Conditions Place Paradigm to test the hypothesis that dopamine antagonism can attenuate the reinforcing properties of LC stimulation. Animals were pretreated with pimozide (0.0, 0.5, 1.0 mg/kg) prior to exposure to one of two distinctive environments paired with rewarding LC stimulation. While LC stimulation resulted in a 10 day preference for the stimulation-paired environment, this effect was dose-dependently attenuated by pimozide pretreatment. Since pimozide alone does not result in place aversions, it was hypothesized that dopamine neurotransmission may be important for the brain stimulation reward resulting from activation of cell bodies even far removed from dopaminergic fiber systems.
489.13

AUTOTITRATION SELF-STIMULATION ANALYSIS OF INDIVIDUAL DIFFERENCES IN THE RAT’S RESPONSE TO AMPHETAMINE, B. Neill and N. Pucetti, Dept. of Psychology, Emory University, Atlanta, GA 30322.

It has recently been reported that it is possible to predict the “vulnerability” of self-administration of amphetamine by their locomotor response to a novel environment. Rats showing a high locomotor response (“high responders”) will acquire self-administration at doses below those necessary for “low responders.” We examined the reaction of “high responders” and “low responders” to amphetamine using medial forebrain bundle/lateral hypothalamic self-stimulation. Rats were selected on the basis of their 1 hr locomotor response to a novel environment (Digiscan activity device). Those in the top and bottom one third of the activity distribution were implanted with a bipolar electrode in the lateral hypothalamus. All were trained to self-stimulate using the autostimulation procedure where intensity dropped every 5 responses.

489.14

CHRONIC DESMETHYLIMIPRAMINE ADMINISTRATION ATTENUATES STRESSOR INDUCED ICSS ALTERATIONS FROM THE PREFRONTAL CORTEX. C. Wolfe and R. M. Zacharko, Department of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6.

Uncontrollable stressors have been found to induce a decline in responding for previously rewarding intracranial self-stimulation (ICSS) from a number of mesocorticortical sites. The stressor provoked attenuation of the rewarding properties associated with electrical brain stimulation has been proposed to be reminiscent of the anhedonic evidence in clinical depression. In the present study, ICSS was assessed from the prefrontal cortex in CD-1 mice immediately, 24-hrs and 168-hrs following exposure to uncontrollable footshock. Marked reductions in ICSS rates were observed in all mice immediately following stressor exposure. Animals in which the ICSS deficits were still evident 1-week post stressor were administered either saline or desmethylimipramine (DMI) twice per day for a 20 day period. Chronic DMI administration ameliorated the stressor induced deficits in ICSS from the prefrontal cortex. Potential explanations for the stressor provoked variations in ICSS and the effects of DMI are discussed.

489.15


The purpose of this investigation was to characterize selected metabolic, cardiovascular and hormonal responses to reinforcing intracranial self-stimulation (ICSS) of the ventral tegmental area (VTA) in rats. Ten Sprague-Dawley rats were stereotaxically implanted with bipolar electrodes aimed at the VTA, origin of the dopaminergic mesolimbic projection, and trained to lever-press for ICSS. They were periodically exposed to the environment a metabolic (operant chamber) for 30 min/d, 5 d/wk for 12 wks during which they were connected to the electrode cable but did not lever-press. At the end of 12 wks, all rats were instrumented with indwelling arterial catheters and performed a 30 min lever-pressing session in the metabolic operant chamber. Oxygen consumption, heart rate, blood pressure and metabolic activity were recorded with the onset and continuation of ICSS over 30 min (p<0.05). Plasma norepinephrine, epinephrine and corticosterone increased significantly over resting values (p<0.05). The results suggest that ICSS elicits heightened sympathetic, metabolic and neuroendocrine activity. These responses provide important information for studies that use ICSS as a motivator of behaviors, such as physical exercise in rats. They also provide valuable information about the physiologic consequences that may be related to inappropriate motorizing behaviors, such as addictive drug abuse.

489.16


1Systems Research Laboratories, 2Operational Technologies, 3Armstrong Laboratory, Brooks AFB, TX 78235-5301.

Most investigators appear to believe that metal wires, typically used to stimulate the brain or record its electrical activity, cannot be used in animals that will be exposed to radio frequency radiation (RFR). Since RFR could couple to the wires, causing current flow (stimulating the brain) or a local temperature increase (causing a les ion). Following an approved protocol, under aseptic conditions, a twisted pair of insulated nicrome wires (dia. 250 μm) were implanted in the central gray within the brains of rats (AP -6.9, L ±2.9, D 5.76 mm). Stimulating (or lesioning) this site causes a species specific withdrawal response (WR). After recovery, the electrical stimulation threshold was determined and the rats were exposed to week long bursts of RFR in the near- and far-fields. During the RFR exposure rats did not display the WR, but did display thermoregulatory behavior (spreading saliva on face/ears). The rats did display WR when stimulated during RFR exposure. The stimulation threshold did not change after exposure, which suggests that exposure did not cause a lesion; this was confirmed histologically.

490.1

MANDUCA NEURAL ANTIGENS REVEALED BY ANTIBODY TO DROSOPHILA PERIOD PROTEIN. Kathleen K. Siwicki and Jessica L. McGrew*, Biology Department, Swarthmore College, Swarthmore, PA 19081.

The period (per) gene of Drosophila melanogaster regulates the periods of behavioral circadian rhythms. An antibody to the per protein was used previously to localize its expression to specific cellular sites in the fly nervous system, and to monitor a circadian rhythm in the protein. (Siwicki et al., 1988). More recently, Zer, Z. et al., (1990, J. Neurosci. 10: 2749-2760). We have used the same antibody to study per-related proteins in a larger insect, the hawkmoth Manduca sexta, with the long-term objective of investigating the effects of per on cellular physiology. In Manduca larvae, anti-per staining was found in 2 pairs of large (40 μm) dorsocentral brain neurons. In adult moth nervous systems, a variety of cellular sites were labeled, including nuclei of many small ventral cell bodies, cytoplasmic granules in some large neurons, and glial nuclei in neuropil regions. Many aspects of this staining pattern are reminiscent of the distribution of the per protein in Drosophila. We are now extending these studies to developing adults, in hopes of finding structures that might be involved in the circadian gating of eclosion.

Also, in immuneblots with protein extracts of moth brains, 44 kD and 110 kD per-like antigens were detected. We are now looking for daily rhythms in the levels of these antigens as a possible indication of functional homology with per.

Supported by NSF grant BNS-9010691 and BNS-9057073, and the Swarthmore College Faculty Research Fund.

490.2


Inst. of Zoology, TU Munich, D- 8046 Garching, Germany, Dept. of Entomology, Univ. of California, Berkeley, CA 94720, Biology Dept. Swarthmore College, Swarthmore, PA 19081.

The cricket Teleogryllus commodus shows a clear circadian rhythm of its calling song. We have stained sections of the CNS of Teleogryllus with an antibody against the Drosophila period (per) protein, which has been used previously to label the sites of per expression in Drosophila and putative circadian pacemaker neurons in the eyes of Aplysia and Bulla. Singing male crickets were sacrificed at four time points of a 12h:12h light-dark cycle. The brain with the retrocerebral complex, the subesophageal ganglion, and occasionally the ventral nerve cord were fixed in 4% paraformaldehyde. Sections were stained with affinity-purified anti-per antibody and avidin-biotin-IRIF method.

The anti-per antibody consistently labelled two somata (approximately 20 μm in diameter) on each side of the lateral protocerebrum, with their axonal branches in all areas of the CNS (including the optic lobes, the mushroom bodies, and the central body). Two fibers project via each NCCII into the corpora cardiaca, the cell bodies and the corpora cardiaca, the intensive staining was independent of the phase of the light-dark cycle or the cricket’s state of activity. But in the labelled projections throughout the CNS, staining was distinctly lower at “lights-on” than at other times of day, suggesting that they may be involved in daily rhythmic functions.

490.1M

BIOLOGICAL RHYTHMS AND SLEEP IX

SOCETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
490.3 AN ANTIBODY AGAINST AN APLYSIA EYE-SPECIFIC 48K PROTEIN RELATED TO THE PERIOD-PROTEIN OF PROSOPHILA SPECIFICALLY STAINS THE APLYSIA RETINA AND OPTIC PROJECTION INTO THE BRAIN. S. Street and J.W. Jacklet, Dept. of Biology and Neurobiology Research Center, SUNYA, Albany, NY 12222.

We have partially sequenced the 48k protein, the major Aplysia eye antigen recognized by a Prosophila period-protein antibody (Neuron 3:51-58). Preparative 2-D gel electrophoresis followed by V8 digestion and sequencing yielded a 20 mer and a 10 mer sequence. Sequences are not similar to the period-protein but the short sequence shows homology to cAMP-dependent protein kinases. By metabolic labeling, we have found that the 48k protein is synthesized in the retina and transported to the distal photoreceptors. Subcellular localization experiments show that 48k is likely to be associated with the cytoskeleton or cell surface. An antibody raised against the long sequence recognized protein of 476-48k, PI 5.9-6.1 spots on 2-D Westerns, and on Aplysia tissue sections stain fibrous structures in the retina and optic nerve. Retinal somata are not stained. The projections of retinal afferents into the cerebral ganglion stained, revealing extensive terminal fields of afferents in the "optic ganglion". Some stained fibers cross over to the contralateral half of the cerebral ganglion, or follow the tentacular and other nerves. Other fibers form tracts and terminal areas in the ganglion. Some continue to the pleural and pedal ganglia and beyond to the abdominal ganglion. These results agree with previous optic tract tracing studies using tritiated markers (Neuron 5:3214) and identify the putative primary brain synaptic areas of retinal afferents. Localization of anti-48k on the EM level is being done. The visual system of Bursatella, a close relative of Aplysia, is stained by anti-48K, but not the visual system of Bulls, a more distantly related ophidian branch. (Supported by NSF grant BNS 881973 to JWJ.)

490.5 POTASSIUM CHANNELS MEDIATE CIRCADIAN CONDUCTION CHANGES IN PAMACEMAKER CELLS OF BULLA S. Michel and G.D. Block, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

The eyes of the marine snail Bulla express a circadian rhythm of compound action potentials (CAP) in vitro driven by endogenous changes in membrane potential of basal retinal neurones (BRN), which are believed to be the pacemaker cells. Intracellular recordings and conduction measurements reveal circadian and light-induced changes in membrane conductance of the BRN. We used the current clamp technique to measure the current-voltage relationship of the cell membrane. Before dawn (circadian time = CT 23) the conductance of BRNs decreased following depolarization of the cell membrane. During the early subjective night (CT 13-15) the conductance increases again and the membrane potential returns to hyperpolarized values (-65 mV). Bath application of the K+ channel blocker TTX (1 μM) at CT 20 led to a reversible decrease of conductance and a depolarization of the cell membrane by 10 mV. The current-voltage curve in TEA at CT 20 is similar to the one measured after dawn (CT 3) in normal seawater. When TEA is given at CT 3 the K+ channel blockers like TTX and 4-Ap and Quinidine to describe the pharmacology of this inward rectifier in order to further characterize and identify the type of channel, which probably is an essential part of the output pathway of the circadian pacemaker in Bulla. Supported by NS 15264 to GDB and DFG-MI3281-1 to SM.

490.7 OPSIN-LIKE IMMUNOREACTIVITY IN THE PUTATIVE PACEMAKER NEURONS OF THE BULLA EYE: M.E. Gesi, R.G. Foster, M.D. Lawrence, and G.D. Block, Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

A circadian rhythm in the frequency of compound action potentials in the optic nerve of the mollusk Bulla nullifica is believed to be generated by the basal retinal neurones (BRNs) of the eye. Light responses from the BRNs persist in eyes with the distal photoreceptor layer surgically removed, and the circadian rhythm can still be phase shifted by light. In addition, artificial seawater with low [Ca2+]+ and high [Mg2+]+ fails to block the light responses from the basal retinal neurons (BRNs) of the eye. Light responses from the cryoprotection, sectioning at -20°C, and treatment with normal goat serum suggest possible differences between the photopigments of the BRNs and other retinal afferents. BRNs were stained with the diaminobenzidine (DAB) peroxidase product. In a second group, desheathed retinas were treated with the antibody (NGS). In a third group, desheathed retinas were treated with the antibody against the long sequence of Bursatella. a close relative of Aplysia, is stained by anti-48K, but not the visual system of Bulls, a more distantly related ophidian branch. (Supported by NSF grant BNS 881973 to JWJ.)

490.4 TYROSINE PHOSPHORYLATION AFFECTS THE CIRCADIAN PACEMAKER IN THE EYE OF THE MARINE SNAIL BULLA GOLDIANA. M.H. Roberts and J.A. Touster, Department of Biology, Clarkson University, Potsdam, NY 13699.

Previously we have shown that inhibition of kinase activity lengthens the period of the circadian rhythm recorded from the isolated eye of Bulla goldiana (Roberts, et al., 1989). We now report the results of additional inhibitor studies.

The circadian rhythm in optic nerve impulses is unaffected by chronic treatment with okadaic acid (0.1 μM), a specific inhibitor of protein phosphatases in vivo (Haystead et al., 1989). This raises the possibility that serine/threonine phosphorylation/dephosphorylation is not involved in rhythm regulation. In order to test this possibility we examined the effects of phenylarsine oxide, a tyrosine phosphatase inhibitor (Bernier et al., 1987), reversibly blocks impulse activity (5-100 μM). In spite of the interpretive difficulties caused by the irreversibility of the treatment, we have further explored the role of tyrosine phosphorylation with genistein, a reversible inhibitor of tyrosine kinase activity (Akiyama, et al., 1987). Chronic moderate doses of genistein (11 μM) elicit impulse activity; upon drug removal impulses return as do light responses, although the circadian rhythm is absent. At lower doses (3.7 μM) period is lengthened while even lower doses (0.37 μM) have no effect on the rhythm. Genistein's actions a/e phase dependent. When applied from CT 2-6 phase delays (-3.0 hrs) are induced. From CT 22-6 the rhythm is damp and phase advanced (+1.5 hrs) or completely eliminated, although impulses and light responses remain. Taken together, these results suggest that tyrosine phosphorylation/dephosphorylation plays a role in circadian rhythm regulation in the Bulla eye. Supported by M25572 to MR.

The crayfish visual circadian rhythm (CCR) is entrained by light. The CCR of the lateral compound eyes consists of an anterior and a posterior photoreceptor, called the caudal photoreceptor (CPR) and lateral compound eyes (LCE) respectively.

To determine the role of the CPR in the CCR, we used a 24-hour light-dark cycle (L:D) and constant temperature and darkness to entrain the CCR. The CPR was isolated from a group of 10 adult crayfish maintained previously in a binocular Photostimulus (PP), which consists of a constant light signal of 4 h duration, and a delay phase-shift following DRB treatment. Overall protein synthesis as measured by leucine incorporation was not affected during a 2h DRB pulse. However, using 2D gel electrophoresis, we found that DRB altered the incorporation of leucine into a small number of proteins.

Some of these proteins may mediate the effects of DRB on the rhythm. A more detailed role for transcription and translation in the regulation of the oscillator will require the identification and study of these important proteins and their mRNAs.

490.10 TRANSCRIPTION AND TRANSLATION APPEAR CLOSELY COUPLED IN THE OCULAR CIRCADIAN SYSTEM OF Aplysia. C. Koumenis*, B. Paik, and A. Eskin. Dept. of Biochem. and Biophys. Sci., Univ. of Houston, Houston, TX. 77204

A model of the circadian system in the eye of Aplysia suggests that proteins are elements of the oscillator mechanism. Regulation of the synthesis of these proteins can occur at the transcriptional or translational level, or both. Translation inhibitors such as cycloheximide (CHX) have been shown to phase-shift the rhythm and also to block the effects of DRB.

Previously, we have shown that pulse treatments of isolated eyes with the reversible transcription inhibitor 5,6,7,8-tetrachlorobenzimidazolodazole (DBR) phase-shift the rhythm, while continuous treatments of DRB lengthen the free-running period of the rhythm. Camptothecin (a transcription inhibitor with a different site of action from DBR) also caused phase-shifts and period lengthening.

To further investigate the roles of transcription and translation, we looked for proteins whose synthesis was affected by DRB. Overall protein synthesis as measured by leucine incorporation was not affected during a 2h DRB pulse. However, using 2D gel electrophoresis, we found that DRB altered the incorporation of leucine into a small number of proteins. Some of these proteins may mediate the effects of DRB on the rhythm. A more detailed role for transcription and translation in the regulation of the oscillator will require the identification and study of these important proteins and their mRNAs.


As the crayfish processes the visual information of environmental movements and shapes, an instantaneous dim in the last animals but not in the rest suggests contralateral modulatory influences which travel throughout subesophageal ganglion.
491.1

EFFECTS OF FEEDING SCHEDULES ON LABELLED 2-DEOXYGLUCOSE UPTAKE IN RAT BRAINS. G.J. Coleman, J. Redman Dept. Psychology, La Trobe Univ. Bendoura 3083, Australia.  The effects of feeding schedules on 2-DG uptake in the brains of rats exposed to either a feeding schedule or to random feeding are described.  Random-fed rats showed substantial disruption of wheel-running activity while meal-scheduled rats showed the usual activity pattern in the dark.  In the brain areas analyzed, differences between treatments were only observed in the SCN and VMH where random-fed rats showed lower levels of 2-DG uptake than did meal-scheduled rats.  These results are preliminary, but suggest that there is no evidence yet for an anatomically distinct site for a food entrained oscillator.

491.3

CHANGES IN PROLACTIN AND CORTICOSTERONE ASSOCIATED WITH PUBERTY AND PHOTOPERIOD IN THE COLLARED LEMMING, Dicrostonyx groenlandicus. B. Sione*, B. H. Navy and R. M. Stegeman. School of Life & Health Sciences, Univ. of Delaware, Newark, DE 19716 (DG, BMW) and Dept. of Biological Sciences, Univ. of Utah, Salt Lake City, UT 84112 (TRM).

The effect of photoperiod on the onset of puberty and associated neuroendocrine changes was examined in male collared lemmings, Dicrostonyx groenlandicus. Animals were gestated in either 16:8 or 8:16 photoperiod from the 16:8 photoperiod were switched to either a 20:4 or an 8:16 photoperiod at weaning (30 days of age). Animals from the 8:16 photoperiod remained in 8:16 throughout the experiment. At 33, 43, and 48 days of age, groups were sacrificed and individuals examined for body, testes, and seminal vesicle weights and the presence of epididymal sperm. Serum was collected and later analyzed for prolactin, corticosterone, and testosterone.  Prolactin occurred significantly earlier, by 5 days, in the 20:4 group than in the group switched to 8:16, the group raised in 8:16 was not different from the other 2 groups. Testes and seminal vesicle weights were similar in the 20:4 group and the group under 8:16, while those animals switched to 8:16 had significantly lower tissue weights. Serum prolactin levels were significantly depressed by shortening day length (both 8:16 groups), as was serum corticosterone. These data suggest that, although significant, the effect of photoperiod on reproductive development is slight. Photoperiod does have, however, influence the neuroendocrine system during the perinatal period. The low levels of prolactin observed in the 8:16 animals suggest that this hormone is not important for reproductive development, but may play a role in the annual pelage cycle.

491.5

KNIFE CUTS DORSAL TO THE PARAVENTRICULAR NUCLEUS (PVN) PREVENT TESTICULAR REGRESSION BUT NOT THE EFFECTS OF SHORT DAYS ON PROLACTIN SECRETION IN HAMSTERS. A.A. Knife. Dept. of Psychology, Monash Univ. Clayton 3168 Australia.  The anatomical location of the postulated food-entrained oscillator in the hamster has not been identified. This study was designed to compare %labelled 2-DG uptake in the brains of rats exposed to either a feeding schedule or to random feeding. Rat brains that were maintained on either a random or fixed 2-hr daily meal schedule under an LD cycle for 59 days. Rats were then killed, their brains frozen then sectioned, filled and subjected to densitometric analysis.  Random-fed rats showed substantial disruption of wheel-running activity while meal-scheduled rats showed the usual activity pattern in the dark. In the brain areas analyzed, differences between treatments were only observed in the SCN and VMH where random-fed rats showed lower levels of 2-DG uptake than did meal-scheduled rats.  These results are preliminary, but suggest that there is no evidence yet for an anatomically distinct site for a food entrained oscillator.

491.2

SUPRACHRASMATIC NUCLEI AND PHOTONUCLEIC TESTICULAR DEVELOPMENT IN SIBERIAN HAMSTERS. K.Kelly*, J.Dars, S.I.Zucker. Dept. of Psychology, University of California, Berkeley, CA 94720.

Light influences the mammalian mammalian neuroendocrine system by altering the duration of pineal melatonin secretion. The suprachiasmatic nuclei (SCN) is a melanin-tanpigmented tissue involved in the neural innervation of the pineal gland. The present study assessed whether changes in SCN activity beginning several days after animals received a single inductive light stimulus are essential for gonadal growth in photoregulated Siberian hamsters. A single long day at 19 days of age produces sustained testes growth over the course of the next two weeks in male animals maintained in short daylengths. At 35 days of age testes weights of light-treated hamsters were significantly heavier than those of untreated animals (143±43 vs 31±4 mg). Hamsters that sustained complete ablation of the SCN 3 days after the light treatment had testes weights of 29±1±5 mg (P<0.001 relative to untreated controls), those that received SCN lesions only, had testes weights of 147±45 mg. These results indicate that changes in SCN neural activity are not necessary for photic stimulation of gonadal growth once the light stimulus has been perceived and neuroendocrine changes in the SCN are likely to be a consequence of SCN damage, including hyperprolactinemia, which might account for the greater than normal testes weights of light-stimulated hamsters with SCN damage.

491.4

DO SIBERIAN HAMSTERS RESPOND TO CHANGING PHOTOPERIODS? M.I. Tubbins, E.L. Meyer, S. Matsumoto and E.L. Bitman. Dept. of Zoology and Program in Neuroscience & Behavior, Univ. of Massachusetts, Amherst 01003.  Photoperiodic cues regulate the development of reproductive and body weight responses of Siberian hamsters to intermediate daylengths after birth. When pregnant dams are maintained in 12L:12D and transferred with their litters to 14L:10D at birth or 15 days later, their offspring mature rapidly. Puberty is delayed in pups gestated in 16L:8D but moved to 14L:10D after birth. In nature, however, pups are exposed to increasing or decreasing photoperiods rather than constant 14L:10D. We tested the possibility that steadily changing postnatal daylengths would override gestational cues.  Beginning on postnatal day 15, pups gestated in 8L:16D or 12L:16D were exposed to constant 14L:10D (groups A and D, respectively), or to daylengths which decreased 8.57 min/day from 16L:8D to 14L:10D (groups C and F) or increased at the same rate from 12L:10D to 14L:10D (groups B and E). Body weight and testis dimensions were measured at laparotomy on day 30. Pups were then held on 14L:10D until sacrifice on day 35.

As expected, postnatal exposure to 14L:10D induced larger final body, epididymal, and testes weights in pups gestated in 16L:8D than those gestated in 12L:12D (groups A vs. B, p<0.01). Gestational daylength also determined responses to steadily increasing or decreasing photoperiod (groups B vs. E and C vs. F, p<0.01). In general, pups responded more to the absolute amount of light than to the direction of photoperiod change. Increasing daylengths were no more inducing than constant 14L:10D. Decreasing daylengths stimulated less testis growth, however, than constant 14L:10D in pups gestated in 12L:12D (groups D vs. F, p<0.01). The results suggest that photoperiodic threshold might be set close to gestational photoperiod and/or that a period of particular sensitivity to daylength may occur shortly after day 15. (Supported by NIMH RO1-44132.)

491.6


The role of the hypothalamus in the modulation of neuroendocrine responses to photoperiod was investigated in Turkish hamsters via placement of horizontal knife cuts at the paraventricular nucleus (PVN) region. Adult male hamsters maintained under a long-day (16L:8D) photoperiod received knife cuts aimed either ventral to the PVN (VKC) or dorsal to this nucleus (DKC), or sham surgery (SHAM). Following surgery, approximately half of the animals in each group were transferred to a short-day (8L:16D) photoperiod. Blood samples and testicular measurements were taken at various times over a 16 week period. The testes were then removed and weighed, the brains prepared for histological evaluation of the lesion sites, and the serum assayed for prolactin (PRL) and follicle-stimulating hormone (FSH).

Similar to the effects of pinealectomy in this species, VKC lesions induced a significant decrease in testicular size, as well as accompanying declines in circulating PRL and FSH levels. Under a short day, VKC lesions induced a decrease in testicular size, but no significant changes in circulating PRL or FSH levels. Similar to the effects of VKC lesions, DKC lesions induced a decrease in testicular size and circulating FSH levels. In contrast, DKC lesions induced an increase in circulating PRL levels. Support for the importance of PVN projections to the brain was obtained by demonstrating that lesioning the PVN blocks testicular regression without disrupting photoperiodic modulation of testes weight. Supported in part by grants BNS890876 to A.A.N. and HD31588 to L.L.B.

Photoperiodic modulation of neuroendocrine activity in the Siberian hamster is dependent upon the integrity of the pineal gland. To further identify the functional correlates of this photoperiodic system in this species, female hamsters received horizontal knife cuts in the hypothalamic aimed between the suprachiasmatic (SCN) and paraventricular (PVN) nuclei. The animals were then transferred to short days and sampled blood and scored for pelage changes at times over a 14-week period. Uteri were then removed and weighed, and the brains prepared for histological evaluation of the lesion sites. PRL and FSH levels were determined via RIA.

The animals were divided into two groups based upon placement of the knife cuts: 1) cuts located ventral to the PVN leaving the SCN intact or 2) through the extent of the SCN. Similar to pinealectomized animals, animals in the PVN group maintained stimulated uteri and high levels of FSH and PRL as compared to control animals. These animals also retained the pigmented peage coloration characteristic of long days. In contrast, animals in the PVN group maintained stimulated uteri and high FSH; however, they showed a marked reduction in PRL and a concurrent molt to the winter-type pelage. These results suggest that not all effects of photoperiod on neuroendocrine activity in this species are mediated directly by the pineal gland, and that the SCN region of the hypothalamus may contain an independent, non-pineal neuroendocrine pathway that is responsible for the stimulatory modulation of PRL, but not FSH, release.


The neuronpeptide LHRH, secreted in a pulsatile manner in the basal hypothalamic region, stimulates the release of LH by the pituitary. Although the secretion of LH is known to be modulated by sensory information from both visual and chemosensory origin, the anatomical pathways involved in relaying this input to the LHRH containing neurons and fibers have been shown to be diffusely distributed in several diencephalic regions. Teleostochronic demonstration of LHRH, combined with anterograde tracing methods, was used to examine the possibility of direct sensory input to the LHRH neuroendocrine population. Intraocular injections of tritiated amino acids or CT-HRP, in both primates and non-primates, showed that several structures containing LHRH positive cells and fibers also receive retinal projections: mediobasal region, anterior and lateral hypothalamus, subparaventricular zone, medial septal nucleus, olfactory tubercle. Simultaneous injection of the anterograde tracer fluoro-ruby in the main and accessory olfactory bulbs showed converging visual and chemosensory projections in the BNST, lateral hypothalamus and basal telencephalon. Injections of retrograde tracers into the BNST and lateral hypothalamus confirmed that projection fibers mainly arise from the accessory olfactory bulb.

These convergent pathways could thus provide an anatomical basis for a monosynaptic relay of visual and/or chemosensory information to LHRH neurons. Work is currently in progress to confirm this hypothesis at the ultrastructural level. This sensory neuroendocrine convergence would be functionally analogous to the olfacto-renaluis system of teleons, in which the LHRH neurons directly connect the retina with olfactory bulb.

NEUROETHOLOGY: ARTHROPODS

492.1 ACTIVITY OF DESCENDING BRAIN NEURONS IN WALKING CRICKETS. H.Bohm* and K. Schilberg, MPI I. Verhaltensphysiologie 8130 Seewiesen, Germany.

In order to study the neural mechanisms involved in the course control of cricket's walking behavior, we recorded intracellularly from descending brain neurons (DEBs), while the animals walked on an air-supported sphere and orientated towards various stimuli like calling song, moving gratings or air streams directed to the cerci.

DEBs responding to only one of the presented stimuli often showed a selectivity for the direction of the stimulus and were in most cases not influenced by walking activity. DEBs responding to all tested stimuli showed in most cases strong habituation, no directional selectivity and a strong influence of walking on their activity.

Moreover, the two main types of DEBs respond to sensory aspects of walking. The activity of one DEB was increased before walking started and was linearly correlated with the forward but not with the turning speed of the animal. Another DEB seems to be a command neuron initiating walking. Hyperpolarizing the neuron immediately stopped walking while depolarizing above a certain firing rate always elicited walking. Further enhancing the firing rate did not produce faster walking and there was no correlation of the neuron's activity and the animal's turning tendency.

492.2 THE BLASING EFFECT OF PRIOR MATE OR COMBAT EXPERIENCE ON COURTSHIP AND AGGRESSION IN THE CRICKET, GRYLLUS BIMACULATUS, S.A. Adams and B.B. Hsu., Dept. of Neurology and Behavior, Cornell University, Ithaca, N.Y. 14853.

In crickets, naive, sexually receptive males normally respond to tactile contact from conspecific males with aggressive behavior, but will copulate similarly presented females. A male cricket's response to conspecifics is not fixed, however, but varies depending upon previous exposure. 15 min. after an aggressive encounter, both winning and losing males exhibited increased aggressive behavior towards sexually receptive females (p<0.05, G=3.85, n=29 males). A losing male also required more time and more contact with a sexually receptive female before it would begin to court (p<0.004, Wilcoxon, n=19 males). Previous aggressive encounters did not inhibit courtship initiation in winning males. Losing males were also less likely to win their next aggressive encounter (15 min. later) even if they were paired with a smaller opponent (p<0.01, G=6.5, n=35 pairs). Losing males, unlike naïve or winning males, fled from the tactile contact of other males (p<0.001, G=8.8, n=35).

Immediately after courtship, males tended to respond to male tactile contact with courtship song (p>0.001, G=17.22, n=35), at least initially. 15 min. after a bout of courtship, males showed an enhanced response to tactile contact with females, requiring less time to initiate courtship (p>0.001, Wilcoxon, n=16 males).

These results suggest that a cricket's prior behavioral performance can result in physiological changes lasting many minutes which alter its behavioral response to tactile contact from conspecifics. Current experiments are exploring the possible role of neuromodulators such as serotonin and catecholamine in mediating this change.


Tethered flying crickets make steering movements away from pulses of ultrasound. Waves of ultrasound can include forewing flap, abdomen swing, and metatoracic leg swing, all of which are lateralized relative to the sound source. A bilateral pair of aching prothoracic auditory interneurons (int-1) has been shown necessary and sufficient to elicit these responses: stimulation of an int-1 (electrically or by ipsilateral ultrasound) causes steering to the contralateral side, whereas hyperpolarizing int-1 results in steering away from the source. To correctly localize a sound source in the presence of echoes, directional information must be taken only from the first-arriving wavefront (precedence effect) and subsequent waves ignored (echo suppression).

Using EMGs of metatoracic leg extensors as an indicator of turning, previous work in our lab showed that the precedence effect and echo suppression occur for delays of 4 to 7.5 ms between a pulse of ultrasound and a simulated echo. For shorter delays, a random turn is made, while longer delays give rise to separate turns in response to each pulse of ultrasound. Bilateral recordings from the two int-1s show no difference in spike number or frequency between the int-1 receiving the first pulse and the one receiving the echo. Therefore, the behavioral effects cannot be accounted for by the temporal changes of activity of the two int-1s.

We have begun recording from another pair of prothoracic auditory interneurons, the omega cells. These are thought to be involved in localization of calling song, but also respond to ultrasound. The omega cells are mutually inhibitory, inhibit the contralateral int-1s, and are responsive to a broad spectrum of frequencies.


In response to models of the males calling songs, young (0-2 day-old) female crickets have higher auditory thresholds (60-80 dB) than do older (4-21 day-old) females (45-55 dB). Changes in the firing threshold of the L1 auditory interneuron parallel the changes with age in the phonotactic threshold of these females. In 1-day-old females, topographical application and microinjection of juvenile hormone III (JHIII) demonstrate that JHIII decreases (25-40 dB) both behavioral and neuronal thresholds within several hours, and that the site of JHIII action on neurons and behavior is within the prothoracic ganglion. The results of the JHIII microinjection experiments strongly suggest that the soma of L1 is the locus for this hormonal effect.

Juvenile hormone binding proteins have been isolated from the hemolymph and, apparently for the first time in insects, from protein extracts of nerve tissue of crickets by labeling them with the tritiated photoaffinity analogue for JHIII (FEFDA) or metoprine (MDK), separating using SDS or native PAGE and identifying them by autoradiography. JHIII binding proteins with a weight of about 23 and 36 kDa were isolated from the pro- and metathoracic ganglia. The 38 kDa protein was found in both ganglia, while the 23 kDa binding protein was much more apparent in extracts from the prothoracic ganglion.

If JHIII were to directly influence the response properties of auditory interneurons by regulation of the expression of an appropriate gene(s), then binding proteins should be present in the soma of these neurons. We are presently attempting to localize JHIII binding proteins in these soma.

Two auditory interneurons (L1 and L3) in the prothoracogenic ganglion of the cricket Acheta domesticus, are involved in phonotaxis and have been shown to be influenced by Juvenile hormone III (JHIII). L1's threshold to 5 kHz calling songs decreases as JHIII levels increase either naturally with increasing age or experimentally applied hormone. This change correlates with a similar decrease in phonotactic threshold with age and JHIII application. Transcription inhibitors block JHIII effects. L1's threshold decrease does not show the same correlation in in vivo experiments with the selective changes in response to natural or artificially changing JHIII levels, and correlates with changes in phonotactic selectivity under similar conditions.

We are using in situ hybridization to identify transmitter receptor mRNA present in these cells with the goal of determining whether the regulation of response properties of auditory neurons might involve regulation of the production of their ligand-gated channels. Ganglia containing iso-topically labeled neurons were sectioned (20 um). Sections containing the labeled somata were examined with an ultraviolet microscope and later visualized using a phosphatase detection system. We have demonstrated the presence of alpha-like nicotinic receptor mRNA (Marshall et al. 1990) using three different 2'-mer BrCTP probes. We are presently determining whether changing JHIII levels might influence the expression of mRNAs for nicotinic receptors in these neurons.

492.7 HABITUATION OF ULTRASONIC ACOUSTIC STARTLE IN FLYING CRICKETS. R.R. Hoy and M.L. May, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The acoustic startle response (ASR) is a rapid, highly stereotyped motor reaction to a loud, transient sound. The survival value of the ASR is reflected in its wide phylogenetic distribution among animals that have a sense of hearing, ranging from insects through all vertebrates, including humans. In mammals, the ASR shows classical habituation. Using Drosophila and C. elegans as model systems, we have shown that the ASR is habituated. We are presently determining whether changing JHIII levels might influence the expression of mRNAs for nicotinic receptors in these neurons.

492.9 LOCUSTS IN A FLIGHT SIMULATOR: THE ROLE OF HEAD MOVEMENTS DURING STABLE FLIGHT AND AVOIDANCE MANOEUVRES. D. Roberts, Institute of Zoology, Basel University, Rheinsprung 9, CH-4051 Basel, Switzerland.

Flying insects strongly rely on vision to detect deviation from course and to maintain flight stability. Visually perceived deviations can have diverse causes: 1) aerodynamical disturbances and motor errors, and 2) active steering manoeuvres. The former (unintended) deviations activate the optomotor (compensatory) steering system while the latter (self-induced) do not. How can a locust initiate intentional turns without being misled by the original course by the optomotor system? The latter turns were flown in a flight simulator that electronically converts the steering force of the animal (measured by a torque meter) into the motion of a surrounding visual pattern driven by a servomotor. The locusts were in control of their visual environment (reafference control), and actively stabilized the panorama over long periods of time. Using immunocytochemistry we identified two principal categories of CC-neurons. The former (unintended) and the latter (self-induced) deviations activate the optomotor system. The former deviations activate the optomotor system with the head moved to the side of the turn, while the latter turns are initiated and executed with the head pointed in the direction of the turn. The former deviations activate the optomotor system in response to turns away from the ultrasound source, thus provoking shifts of the panorama that were not compensated by the optomotor response. Compensation and avoidance steering involved the same neuronal effectors, e.g. abdominal and hind wing ruddering and wingbeat modulation. In both cases, ruddering was directed to the side of the turn. During compensatory steering, the head moved to the side of the turn, while it moved to the side away from the turn during avoidance steering. For both reactions, head movements occurred 30-40 ms after torque production began and thus cannot be considered as initiating steering. Head turns during avoidance steering were shown to be critical and independent of the visual feedback. This indicates that compensation and avoidance are involved in the modulation of the steering response, with the head movements being a natural consequence of the sensory input.
VENTRAL GIANT INTERNEURON WIND FIELDS IN THE COCKROACH
Sci. and Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

We are attempting to construct a physiologically accurate model of the escape
system of the cockroach. At last year’s meeting, we presented the basic
components of a model constructed with the back-propagation training
scheme. Our model of the ventral giant interneurons (vGIs) was trained on
the normal wind field responses and constrained by published patterns
of connectivity from cercal afferents (Daley and Camhi, J. Neurophysiol.
60: 1350).

We have now refined this model by incorporating additional training data and
constant constraints. The model has now trained on vectors for normal;
abnormal and animal and cercal afferent responses (Westin et al., J.
In addition, the sigmoidal responses of the model vGIs were fit to firing characteristics
of actual vGI neurons. A series of intracellular experiments characterized current-
frequency relationships of individual vGIIs. Mean number of action potentials
for varying levels of injected current were plotted.

A particularly interesting aspect of this system is the adaptation we observe
within vGI response patterns as a result of unilateral cercal ablation. With
our more physiologically accurate model, we can now simulate this adaptation by
retraining a lesioned model to the recovered wind fields. By comparing
connection strengths before and after simulated adaptation, we hope to gain
insight into possible loci of plasticity.

Supported by ONR grant N00014-90-J-1545 to RDB, NIH grant NS 17411 to
and NSF grant BNS-8610757 to HJC.

EXPANDING THE BOUNDARIES OF THE COCKROACH “EVAPE circuit”: LESIONS AT THE CERVICAL LEVEL ALTERT ESCAPE BEHAVIOR.
A.P. Keegan, E. Mara, and C.M. Corner Dept. of Biological Sciences,
University of Illinois at Chicago, Chicago, IL 60680.

The escape behavior of the cockroach (Periplaneta americana) has been
known for many years to be triggered by wind stimulation of the cercal cilia, and
to involve giant interneurons (GIs) ascending to the thoracic region of the CNS.
We recently reported that pheromoneavad predators appear to be detected by wind cues,
but rather by direct touch (Flc. Neurosci. Abstr. 15: 294) and that some of these tactile
response (e.g. those derived from the antennae) involve novel interneuronal pathways
descending from the head ganglia to thoracic regions (Brain Res. 335: 347).

We have now made lesions between the head ganglia and thoracic circuits
and used high-speed camera to capture the characteristics of the descending
and tactually elicited behavior. We have found that such lesions alter antennal tactile escape responses,
and also modulate cervical wind-evoked escape responses. When one neck
conductive was transected, animals responded less consistently to antennal tactile
stimuli and the directionality of their turning was shifted: they often turned toward
rather than away from stimuli on the side opposite the lesion. Animals with this
response type responded to cervical stimuli at normal levels, but the orientation
of their turning was shifted in the same way as touch.

This demonstrates lateralization of descending tactile pathways for escape,
and also implies that pathways from head ganglia influence how thoracic circuits
use ascending GI signals to initiate turning movements. Our data suggest that
rital modulation of G1-to-motor circuits is not likely to involve a phasic wind-
directed signal, but rather a more tonic signal that can bias premotor and/or motor
cells.

Supported by NSF grant #NSF-89-09051 to C.M.C.

FLIGHT MUSCLE ACTIVITY UNDERLYING PHEROMONE-
V8W 2Y2; 1 Dept. Psych., U. of Leth., Lethbridge, AB T1K 5M4.

Male M. sexta approach females for mating by flying a characteristic side-
to-side zigzagging pattern. This upwind pattern results from self-steered maneuvers
initiated and modulated by wind-borne pheromone released by the female.

To characterize motor patterns underlying flight maneuvers, we have recorded electromyograms (EMGs) from flight muscles of M. sexta males flying freely in kinesio film. EMG activity with a video
record of the zigzagging flight track in a laboratory wind tunnel (closed-loop conditions)
We have also recorded the activity of these muscles, in the same
dimensional individuals, during flight. Flight data acquired by video and
photographically stimulated or pheromone-stimulated (open-loop conditions).
We recorded from three muscles: the left dorsal longitudinal muscle (DLM), a main wing-depressor; and the left and right third abdominal long muscles (ALMs), which have been identified as "steering" muscles in
crabs held in water were videotaped from the side and below, and analyzed with EW.

Digging resembles walking in two respects. 1) Contra-lateral legs, including the chela, move out of phase (but become synchronized when the crab is almost dug in). 2) Ipsilateral legs move in a more continuous, stereotyped response to acoustic stimuli. Digging
likely solutions to walking in several ways. 1) Leg 4 plays a special role in
digging and its control may be different from the others. a) the power
stroke of Leg 4 is backwards and does not contribute to forward
movement. b) the sequence of joint movements is proximal-distal in Leg 4, but
distal-proximal in Legs 2 and 3. 2) Most joints move active
movements, in walking, distal segments are largely carried along.
Each leg moves in different directions relative to the body, in forward
walking legs move parallel to the midline. 4) The abdomen and the
chelae move and contribute to locomotion. The abdomen cycles at
about twice the rate of the thoracic legs; its small size and rapid
movement suggest that it acts by "paddling," making the sand more
fluid. Research supported by NSERC.
**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991**

### 493.1 CORRELATION OF APOMORPHINE- AND AMPHETAMINE-INDUCED TURING BEHAVIOR WITH NIGROSTRIATAL DOPAMINE DEPLETION


In the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease, some controversy exists concerning the use of apomorphine (APO) vs. amphetamine (AMP)-induced rotations as indications for nigrostriatal dopamine (DA) depletions. APO produces rotations contralateral to a lesion and is dependent upon the development of receptor supersensitivity, while AMP produces rotations ipsilateral to a lesion due to induction of receptor supersensitivity on the intact side. Our objective was to evaluate if either drug-induced behavior is more predictive of the extent of nigrostriatal DA depletions. Male and female Fischer 344 and Sprague-Dawley rats were unilaterally injected with 6-OHDA (20 micrograms/microliters/4min) into the medial forebrain bundle. The animals were tested twice each on APO (0.05 mg/kg) and AMP (0.5 mg/kg). Following the behavioral tests, right and left striatum (STR) and substantia nigra (SN) were analyzed for catecholamine content by HPLC-EC. The results showed that an animal that had greater than 99% (maximal) depletion of DA rotated well on either APO (500/hr) or AMP (300/hr). However, many sub-maximally lesioned rats were seen to rotate adequately on AMP but not on APO. We believe that APO-induced rotation behavior may be a better predictor of nigrostriatal DA lesions as compared to AMP. (Supported by USPHS grants NS01919 & AG08434)

### 493.2 DOPAMINE UPTAKE IN PREFRONTAL AND CINGULATE CORTEX AFTER CHRONIC AMPHETAMINE TREATMENT

K. A. Segal, G. A. Gerhardt, J. M. Massero. Deps. of Pharmacology & Psychiatry, Univ. of Colorado Health Science Center, Denver, CO 80262.

Amphetamine (3mg/kg) given for 7 days to male rats induces a behavioral sensitivity to a single injection of amphetamine (1mg/kg) seven days after the last injection. In this study we found that in the uptake of dopamine (DA) in prefrontal and cingulate cortex synaptosomes in rats chronically treated with amphetamine compared to saline controls. The increase in DA uptake is not seen 1 day after the last injection. There is also no increase in DA uptake into striatal or nucleus accumbens synaptosomes of chronically treated rats. Amphetamine blocks the uptake of DA in the central and chronically treated NE rats without any apparent change in sensitivity. With 1x10-7 M desipramine (DHE) blocking norepinephrine uptake sites, the increase in DA uptake in cortex was only about 30%. While with 1x10-7 M CBR 12909 blocking DHE, there was a 70% increase in uptake of DA into norepinephrine sites. These data indicate that DA uptake into both DA and NE synaptosomes is increased in the medial prefrontal/cingulate cortex by chronic amphetamine treatment. (Supported by USPHS grants NS04511 and NS09199.)

### 493.3 FUNCTIONAL EFFECTS OF REPEATED APOPMORPHINE ADMINISTRATION

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The repeated administration of apomorphine (APO) produces a progressive enhancement of its effects on motor behavior, in that stereotypic movements become more intense with repeated treatment. Using the 2-[14C]d-glucose method, the distribution of alterations in functional activity associated with the repeated administration of APO was determined and compared to the distribution of changes associated with its acute administration. Male rats were treated with saline or APO (1mg/kg, s.c.) or AMP (10mg/kg), and its effects were contrasted to the action of acetylsalicylic acid (ASA; 160mg/kg). One week later APO or saline was administered and rates of glucose utilization were measured, pre- and post-treated with saline and acute APO, pretreated with saline and treated with APO; and chronic APO, pretreated and tested with APO. The acute APO selectively increased rates of metabolism in areas thought to mediate the actions of APO alone, e.g. the globus pallidus, substantia nigra reticulata, and entopeduncular nucleus, and decreased metabolism in the lateral habenula, as reported previously (cf. McCulloch et al., 1982). In contrast, repeated APO treatment decreased metabolic rates in limbic areas, as well as in the substantia nigra, hippocampus, and prefrontal cortex. Elevations in rates of metabolism, similar to those observed with acute APO treatment, were also seen in the nigrostriatal system, although these increases were somewhat attenuated when compared to the acute changes. The metabolic effects of repeated APO administration, then, are different from those of acute administration, and chronic APO treatment produces different functional effects in the nigrostriatal and mesolimbic systems. Finally, the augmented behavioral response to repeated APO is not paralleled by corresponding increases in functional activity in any brain area examined.

### 493.4 ENVIRONMENTAL ENRICHMENT ALTERS AMPHETAMINE-STIMULATED DOPAMINE RELEASE AND SYNTHESIS IN THE NUCLEUS ACCUMBENS

S. L. Bowling*, J. K. Rowlett & M. T. Bardo, Department of Psychology, University of Kentucky, Lexington, KY 40506.

In two experiments, rats were raised from postnatal day 21 to about day 60 in either an enriched (EC) or isolated (IC) condition. In the first study, amphetamine-stimulated release of dopamine (DA) was assessed in vitro from slices of the nucleus accumbens and striatum. DA and dihydroxyphenylacetic acid (DOPAC) levels were measured by high pressure liquid chromatography (HPLC). EC rats had less total DA and DOPAC than IC rats in the nucleus accumbens, but not the striatum. EC rats released less DA than IC rats when stimulated by amphetamine (1 mg/kg, 10 mg/kg). In the second study, the activity of the mesolimbic DA reward pathway relative to IC animals.

### 493.5 IN VIVO MECHANISM OF ACTION OF DEPRENYL: BIOCHEMICAL AND BEHAVIORAL STUDIES

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In vivo microdialysis was used to concurrently measure the behavioral and striatal dopamine (DA) response following the administration of the irreversible type B selective monoamine oxidase (MAO) inhibitor, deprenyl (10 mg/kg). Treatment effects were compared to the action of an irreversible MAO-A inhibitor, clorgyline (4 mg/kg). Clorgyline administration demonstrated a response indicative of inhibition of MAO-A in that a gradual and enduring increase in extracellular DA and 3-methoxytyramine and a decrease in DOPAC and HVA which lasted for at least six hours. In contrast deprenyl administration produced rapid and transient changes in DA and its metabolites. This pattern of deprenyl effects closely resembled the biochemical response following amphetamine administration (Kuczenski and Segal, J. Neurosci., 9:2051, 1989). Furthermore, our behavioral data demonstrated a stimulant-like increase in locomotor activity following deprenyl which was not observed following clorgyline. Taken together, these data suggest that systemic administration of deprenyl does not augment striatal extracellular DA by irreversible inhibition of MAO-B, but rather produces its effects on behavior and DA dynamics as a consequence of its conversion to amphetamine.

(Supported by DA04157 and DA05689.)

### 493.6 THE ROLE OF DOPAMINE AND CALCULION CHANNELS IN AMPHETAMINE-CONDITIONED LOCUS COERULEUS CONDUCTOMES


Previous research has shown that dopamine (DA) D1 and D2 antagonists cannot block the establishment of amphetamine (AMPH)-induced conditioned locomotion (CL) in rats. However, IC rats, but not IC-D1 or IC-D2 rats, have a reduced functional activity of the mesolimbic DA reward pathway relative to IC animals.

(Supported by USPHS grant DA05312.)
493.7

Our recordings of striatal single-unit activity in freely moving rats showed that amphetamine (AMPH) typically excites motor-related and inhibits nonmotor-related neurons (Haracz et al., Brain Res., 489:366, 1989). Because motor-related cells regularly increase activity during movement, AMPH-induced excitations may reflect a behavioral feedback response, rather than a primary drug effect. This possibility was assessed with a new behavioral clamping method in which rated videotapes were used to match pre-AMPH exploratory behaviors with similar post-AMPH behaviors. Firing rates were compared within each match. AMPH (0.1 mg/kg, sc) significantly increased movement-related activity of motor-related cells, suggesting a direct drug effect in exciting striatal neurons independent of behavioral feedback. This effect is abolished in rats with bilateral ablations of frontal cortex. Thus, AMPH-induced excitations of striatal neurons require intact corticostriatal projections. Preliminary data from striatal nonmotor-related cells, however, indicate that such projections are not required for AMPH-induced striatal inhibitions. These results suggest that AMPH induces dopamine neuromodulation, facilitates or inhibits the activity of striatal dopamine neuromodulation, and inhibits or facilitates striatal motor-related activities. This is supported by USPHS grant DA 02451.

493.8

Two studies were conducted to provide a further characterization of the neurochemical and behavioral effects of phenycyclidine (PCP). The first experiment utilized in vivo microdialysis to assess extracellular dopamine (DA) concentrations after PCP administration into the nucleus accumbens (ACC). Rats were implanted with dialysis probes into the ACC and were tested for two days. Rats received either PCP (0.4 x 10^-4 M dissolved directly into the perfusing medium) for two 45 min sessions, or continued perfusion with the normal medium, in a random order. PCP significantly increased extracellular DA levels. In a second experiment, chronic indwelling cannulae were implanted bilaterally into the ACC. Rats were injected with 1.0 ml of either saline or PCP (30.0 μg or 60.0 μg) in random order on 3 separate days, and placed in a motor activity chamber. PCP caused a dose-related increase in locomotion, with 60 μg causing the largest increases in motor activity. Little or no headwaving or ataxia was observed after injection of PCP. These results suggest that PCP can elevate extracellular DA concentrations by actions on the terminal regions of the DA neurons, and that these effects are related to some of the stimulant properties of PCP.

493.9
THE EFFECT OF INTRA-ACCUMBENS AMPHETAMINE ON LATENT INHIBITION USING AN ON-BASELINE, WITHIN-SUBJECT DESIGN. A. S. Kilcoyne and T. W. Robinson (SPON: Brain Research Association), Dept. of Exp. Psychology, University of Cambridge, Cambridge, CB2 3EB, UK.

Latent inhibition (LI) is a phenomenon in which repeated, non-reinforced exposure to a stimulus elicits subsequent conditioning to that stimulus. This is generally considered to be the result of learning to ignore or not to attend to the preexposed stimulus, and as such LI is commonly used to assess the influence of drugs and lesions on attentional processes. Several recent experiments have demonstrated that low doses of amphetamine, given acutely during preexposure and conditioning, abolish LI in a subsequent test session. These effects have been attributed to amphetamine-induced changes in mesolimbic dopamine (DA) function. The aim of this experiment was to test this hypothesis directly by examining the effects of intra-accumbens amphetamine on LI. In a within-subjects design with rats, LI was mapped across a conditioned suppression procedure. Animals that received bilateral infusions of d-amphetamine (1μl, 10g/ml) immediately prior to preexposure and conditioning sessions showed a single trial retardation in conditioning, but subsequently developed a LI effect comparable to that of animals receiving control infusions of vehicle. These results provide no support for the hypothesis that the mesolimbic DA system plays a role in the attentional processes producing LI. It is likely that the decrements in LI found following acute, peripheral injections of amphetamine are due either to non-specific behavioural effects of the drug, or to effects of the drug on non-mesolimbic systems.

493.10
EXTRACELLULAR MONOAMINE LEVELS ARE DISSOCIATED FROM CHANGES IN LOCOMOTOR ACTIVITY FOLLOWING MEDIAL PREFRONTAL CORTEX ABLATION. E. Castedhe, D. Fazio, G. Millerman and Psychology Arizona State University, Memphis State University & University of Lethbridge, Lethbridge, Alberta T1K 4M4.

Evidence suggests that some of the behavioral changes that follow cortical injury are produced by compensatory changes in non-specific areas of the striatum. We examined the motor activity of rats that had received bilateral medial frontal cortex lesions and also examined extracellular monamine levels in motor cortex and nucleus accumbens using in vivo microdialysis. Medial prefrontal cortex damaged rats displayed an attenuation in locomotor behavior during baseline measures and after an amphetamine injection (1.5 mg/kg, s.c.) one day after injury. In contrast, after two weeks recovery animals displayed heightened locomotion in relation to controls in response to amphetamine. There were no changes in extracellular levels of mesocannethal electric cathecholamines (dopamine, DOPAC & HVA) at any time; and the serotonin metabolite, 5-HIAA, displayed a significant enhancement in nucleus accumbens extracellular levels, but only after an amphetamine challenge one day after the day following medial prefrontal cortex lesions. The implications for non-dopaminergic mechanisms that might be responsible for recovery of behaviors traditionally thought to be mediated by mesocannethal dopamine systems will be discussed.

493.11

The mechanism by which the anxiogenic 8-carbolime FG-7142 reduces exploratory locomotor activity in rats is not known. FG-7142 has been shown to selectively enhance DA release within the medial prefrontal cortex (MPCF) and to reduce SHT transmission in the hippocampus. These findings support the hypothesis that changes in the control of exploratory activity. To assess the anatomical specificity of FG-7142-induced reduction of locomotion, we evaluated its effects in rats with ibotenic acid (6 μg) lesions of MFPC dorsal (DH) or ventral hippocampus (VH) as compared to the corresponding sham-operated animals (infused with buffered saline). We found that FG-7142 suppressed the locomotor behavior in control and lesioned rats. Its effect on locomotor reduction was potentiated in MFPC lesioned rats, but abolished in DH rats. These results suggest a selective role of the DH in mediating FG-7142-induced attenuation of exploration, possibly related to specific serotonergic projections to this region.

493.12
DISCRIMINATORY ACTION OF GABA AND VIGABATRIN ON ISOLATION-INDUCED OFFENSIVE BEHAVIOR IN MICE. M.Y. Hasan, B.D. Sewell, and P.J. Nicholls. SPON: Brain Research Association, Division of Pharmacology, Welsh School of Pharmacy, UWWC, PO Box 13, Cardiff CF1 1XK, UK.

GABA is involved in modulating mood and behavior and inhibition of GABA-transaminase reduces offensive behavioral expression in mice. Vigabatrin is an irreversible inhibitor of GABA-transaminase and increases GABA levels in the CNS. This study compares the effects of vigabatrin with those of other GABAergic behaviors including locomotion, non-social, social and in particular offensive profiles. These behaviors were assessed in male and female mice of similar weight (20-25 g, n=10) using a modified isolation procedure previously described by this laboratory. The behavioral interaction was observed in between 3 weeks at individual and group-housed individuals over a test period of 10 min. Pretreatment with GABA (400mg/kg ip) or Vigabatrin (400mg/kg ip) greatly reduced the incidence of offensive encounters displayed, delayed the onset of offense without modifying social, non-social and locomotor behaviors. Thus GABA and Vigabatrin anti-offensive activity is comparable and occurs at behaviorally selective doses.
493.13
PREVENTION BY MORPHINE OF PENILE ERECTION AND YAWNING INDUCED BY APOMORPHINE AND OXTOCIN.
M.R. Nelles, B. Stancampiano and A. Arigiolas.
B.B. Brodie Dept. of Neurosciences, Univ. of Cagliari, Via Porcell 4, 09124 Cagliari (Italy).

The effect of morphine administered systemically or into the paraventricular nucleus of the hypothalamus (PVN) on penile erection and yawning induced by oxytocin or the dopamine agonist apomorphine was studied in male rats. Systemic morphine (0.5-5 mg/kg i.p.) prevented in a dose-dependent manner penile erection and yawning induced by oxytocin (30 ng i.c.v.) or by apomorphine (80 μg/kg s.c.). Morphine (0.1-5 mg/kg) did not affect CP-593 (5 μg) injected into the PVN 10 min before oxytocin or apomorphine, prevented penile erection and yawning induced by the unilateral PVN microinjection of oxytocin (10 ng) or apomorphine (50 ng). The morphine effect was abolished by naloxone (1 and 3 mg/kg i.p.) 15 min before morphine. The results suggest that morphine prevents apomorphine- and oxytocin-induced responses by inhibiting the activity of oxytocinergic neurons through μ-type receptors in this hypothalamic nucleus. The finding raise the intriguing possibility that opiates inhibit sexual behavior by inhibiting central oxytocinergic transmission.

DRUGS OF ABUSE—AMPHETAMINE AND NICOTINE

494.1
ROTATIONAL BEHAVIOR AND CONCURRENT STRIATAL DOPAMINE OVERFLOW FOLLOWING AMPHETAMINE, PHENCYCLIDINE AND MK-801.
A. Mele, D.J. Fontana and A. Post.
BPR/NIH, Bethesda, MD.

It has been suggested that some of the behavioral and pharmacological effects of PCP, like amphetamine, are determined through increases in dopaminergic (DA) function. The purpose of this study was to compare the effects of PCP, amphetamine, and the NMDA antagonist, MK-801, on rotational behavior and striatal DA overflow in rats with unilateral nigro-striatal lesions. Rats were lesioned in the left MFB with 6-0HDA and implanted with a guide cannula in the striatum contralateral to the lesion. One week following surgery, a microdialysis probe was inserted into the striatum through the implanted guide. The animals were placed in a rotometer which had been modified to allow the collection of dialysate. Levels of DA and its metabolites were measured in the dialysate with HPLC-EC procedures at 20 min intervals. Following stabilization of DA levels, the animals were injected i.p. with either PCP (10 μg/kg, amphetamine (1 mg/kg), or MK-801 (0.25 mg/kg). All three drugs produced significant increases in rotational behavior ipsilateral to the lesion. Only amphetamine and PCP, however, elicited increases in striatal DA overflow. The effect of MK-801 on striatal DA were rather modest (approximately 50% increase over baseline at peak effect), whereas its effect on rotational output was quite dramatic (approximately 80 rotations/20 min at peak effect). Amphetamine, on the other hand, produced a more robust effect on DA (approximately 150% increase at peak effect) at the same time elevating rotational output to only 40 rotations at peak. MK-801 had behavioral effects similar to amphetamine, but did not produce any alterations in striatal DA. Thus, there appears to be a significant dissociation between the effects of these drugs on rotational behavior and their ability to alter striatal DA function. It is suggested that the modest DA actions of PCP contribute little to determining its effects on nigrostriatal functions.

494.2
METAMPHETAMINE-INDUCED STIMULUS PROCESSING DEFICITS.
B.G. Cooper*, A.E. Buitt, D.J. Hardy* and G.K. Hodge.
Psychology Dept., University of New Mexico, Albuquerque, NM 87131.

Neurotic doses of amphetamine (MA) resulted in cognitive deficits in rats tested in an operant discrimination task. Animals were shaped to bar-press and then received four s.i.c.i. injections (2 hours between injections) of either saline or MA (20 mg/kg or 12 mg/kg). One week post-injection, animals were tested in an operant discrimination task. The first phase of the task required animals to press a bar for a food reward during the presentation of a 10-sec target light. A 10-sec period of darkness followed the target light; bar-presses during this period were recorded as errors. During the second phase of testing, a 10-sec distractor light followed the target light instead of darkness. Errors were penalized in this phase by delaying the onset of the target light by an additional 10 sec. During the third phase of testing, the target window was reduced to 3 sec, with distractor light and penalty conditions remaining constant. Results from the second phase of testing showed an initial increase in errors in the 10 mg/kg group, followed by rapid recovery; the 12 mg/kg group showed a more prolonged increase in errors with subsequent recovery. The third phase of testing was characterized by a pronounced increase in errors in both MA groups. Results suggest the inability of MA-treated rats to alter response patterns as task parameters change.

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494.3
MDMA'S EFFECTS ESTABLISH A CONDITIONED PLACE PREFERENCE AND ELICIT SIGNS OF SEXUAL AROUSAL AMONG RATS.
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Rensselaer Polytechnic Institute, Troy, NY 12180.

Studies of methamethedoxymethamphetamine (MDMA) are of interest because of its potential addiction liability and its neurotoxicity. Previously, we have demonstrated that MDMA establishes a positive conditioned place preference (CPP) among rats. Doses of 30 microgram/kg MDL 72222, a selective serotonin 5-HT3 antagonist, and 30 mg/kg MDMA, two compounds that attenuate the release of dopamine, blocked the establishment of a MDMA CPP. The data lead to the suggestion that MDMA's ability to elicit positive affect (and, thereby, positive reinforcement) is modulated by dopaminergic pathways. In observing rats being conditioned with a 6.3 mg/kg dose of MDMA, the ejaculation of seminal plugs was noted. With the first injection, in one experiment, (a) rats receiving saline or 5-HT3 antagonist (56 mg/kg SC, 4 hr before conditioning) almost never left plugs in the place of conditioning, while (b) 7 of 12 rats receiving only MDMA, and (c) 12 of 12 rats receiving MDMA and naltrexone left plugs.

494.4
IN VITRO METABOLISM OF 6-HYDROXY-3,4-(METHYLENEDIOXY)METHAMPHETAMINE TO THE NEUROTOXIC, 2,4,5-TRIHYDROXYMETHAMPHETAMINE.

Since O-dealkylation has been reported as a primary in vivo metabolic pathway for 3,4-(methylenedioxy)methamphetamine (MDMA) in the rat, it is likely that the previously identified aromatic hydroxylated metabolites of MDMA (Lim and Foltz 1990) are further metabolized by O-dealkylation to compounds similar in chemical structure to the potent neurotoxin, 6-hydroxymethamphetamine. Thus, this study examines the in vitro rat liver metabolism of 6-hydroxy-3,4-(methylenedioxy)methamphetamine (6-OH-MDMA) to 2,4,5-trihydroxymethamphetamine (THM). THM was found to cyclize to 5,6-dihydroxy-1,2-dimethylindole (DHDMI) when incubated with rat liver S-9 fraction (Lim and Foltz, 1990). Selected reaction monitoring of the daughter ion at m/z 218 from DHDMI provided a highly sensitive method for the detection of THM. Comparison of the daughter ion profiles corresponding to the in vitro incubate containing 6-OH-MDMA and control indicated the presence of a peak not found in the control. Conclusive identification of this peak as DHDMI was from co-injection with synthetic DHDMI. Additional of SKF-525A, 1-aminobenzotriazole and quinidine resulted in 100, 76 and 100% inhibition of their vitro metabolism of 6-OH-MDMA. This study conclusively identified THM as an in vitro metabolite of 6-OH-MDMA. Furthermore, the metabolism of 6-OH-MDMA to THM is cytochrome P-450 dependent; the specific cytochrome P-450 isozyme P450ID6 appears to be involved. (Supported by NIDA grant 1RO1 DA 05860-01).
Elayan*, M. Johnson, G.R. Hanson, K.H. Lim *, R.L. Foltz* and M. ONOAM INERGIC SYSTEMS IN THE RAT BRAIN. I.M. J.W. Gibb. Department of Pharmacology and Toxicology. University of Utah, Salt Lake City, Ut 84112.

This study was designed to test the effect of a new amphetamine derivative, 2,4,5-trihydroxyamphetamine (THA), on the monoaminergic systems in the rat brain after intraventricular injection. In the long-term, rats were injected with THA (0.25, 0.5, or 1.0 μmole in 20 μl of 0.1% ascorbate in saline). A control group received vehicle alone with the same volume of the vehicle. Hippocampal tryptophan hydroxylase (THP) activity was markedly reduced to 5%, 1%, and 76% of control by 0.25, 0.5, and 1.0 μmole THA, respectively. THP activity in the striatum was decreased to 74%, 81%, and 56% of control and striatal tyrosine hydroxylase (TH) activity was reduced to 67%, 10%, and 19% of control, with the same doses, respectively. Hippocampal and striatal TH activity were decreased to 10%, 18%, and 16% of control. In the short-term experiment, the rats were sacrificed 3 hours after the injection of THA (1.0 μmole) or saline. Hippocampal and striatal TH activity were decreased to 10%, 18%, and 16% of control. This study demonstrates that THA is a potent compound that decreases hippocampal and striatal TH and TH activity when administered intraventricularly. (Supported by USPHS grants DA 00869, DA 04222 and DA 03860).

494.9


Individual vulnerability to the reinforcing properties of drugs of abuse appears to be an endogenous characteristic predisposing to addiction. In rat we have previously shown that individuals with a higher locomotor reactivity and a longer corticosterone secretion to novelty (High Responders, HR), have a higher predisposition to acquire amphetamine self-administration (SA)[Science 1989 245:1551-1553]. Animals with weak behavioral and endocrinological responses to novelty (Low Responders, LR) do not develop SA. In the present study we have investigated whether corticosterone levels have a direct influence on the locomotor response to amphetamine (AMPH). We have shown that naturally resistant to develop amphetamine self-administration where injected with corticosterone they develop the same locomotor response (0.5mg/kg i.v.) administered after 8 days of training for SA (P<0.01), during which resistant animals do not develop SA, and when the hormone (50mg/ml) was presented in the drinking water of LR (P<0.05).

Corticosterone also modified SA of individuals that spontaneously acquired this behavior. In this case, and with both the protocols described above, the hormone decreased the intake of the drug of HR animals. This apparent opposite effect of corticosterone in the two groups of individuals is similar to the phenomenon observed with the compound 5-methyl-4-phenyl-1,2,3,6-tetrahydroisoquinoline. For each injection is increased, there is a faster acquisition of this behavior and a decrease of the number of drug injections during SA. Thus, it is suggested that corticosterone acts on SA by increasing the reinforcing properties of amphetamine. The possible direct reinforcing effect of corticosterone is under investigation and will be discussed.

494.10

THE BEHAVIOURAL EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF 4-METHYLAMINOREX (4-MAX) ON ACTIVITY IN THE RAT. Buttsch, K., Ashby, C. R. Jr., Pan, H. S., Kimura, C., Schwartz, J., and Wang, R. Y. Department of Psychiatry, SUNY Stony Brook, Putnam Hall, South Campus, Stony Brook, N.Y. 11794-7970.

The compound 4-methylaminorex is a phenylpropionic derivative with 4 isomers; 4S,4R, 4S,4S, 4R,4S, and 4R,4R. The 4R,4S isomer produces sympathomimetic, stimulant, and anorectic effects similar to amphetamine and psychological effects similar to amphetamine and cocaine. We show here that acute and subacute administration of 4R,4S isomer at 3 mg/kg, reduced the locomotor response to amphetamine (AMPH) in all the groups. In the present study, we investigated whether corticosterone levels have a direct influence on the locomotor response to amphetamine (AMPH). The highest locomotor activity of HR animals seems to be partially dependent on the level of cortical corticosterone. Thus, the addition of corticosterone (50mg/ml) to the drinking water of LR increased their nocturnal locomotor activity. The drug effect for the dose 5mg/kg was more pronounced in LR rats, which also have a higher predisposition to acquire amphetamine self-administration (SA). The HR animals also have a higher locomotor response to amphetamine (AMPH) than HR animals. Thus, the addition of corticosterone (50mg/ml) to the drinking water of LR increased their nocturnal locomotor activity. The drug effect for the dose 10 mg/kg was more pronounced in LR rats, which also have a higher predisposition to acquire amphetamine self-administration (SA). The HR animals also have a lower affinity of type II receptors in the hippocampus (P<0.05) and amygdala (P<0.01) and a reduced affinity of type II receptors only in the hippocampus (P<0.05). The receptors in hippocampus were not different in the two groups. These data suggest that a reduced affinity of corticosteroid receptors in hippocampus and amygdala may be responsible for the higher locomotor response to stress observed in the HR animals. Thus, it is suggested that pharmacological manipulations of corticosteroid receptors may open new therapeutic strategies for compulsive drug use.

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494.5

EFFECT OF 2,4,5-TRIHYDROXYAMPHETAMINE ON MONOAAMINERGIC SYSTEMS IN THE RAT BRAIN. L.M. Elayan*, M. Johnson, G.R. Hanson, K.H. Lim *, R.L. Foltz* and J.W. Gibb. Department of Pharmacology and Toxicology. University of Utah, Salt Lake City, Ut 84112.

Recently, we suggested that reduced corticosterone feedback in the rat may induce a longer secretion of corticosterone after stress, also increase likelihood differences in amphetamine SA and characteristics of corticosteroid receptors in hippocampus, amygdala and hypothalamus. Rats were selected on the basis of reactivity to novelty and desensitization[1]. High Responders (HR) rats, with high locomotor reactivity, prolonged corticosterone secretion in response to novelty, and fast acquisition of amphetamine SA; 2) Low Responder (LR) rats, with low locomotor reactivity and short corticosterone secretion in response to novelty, fail to acquire amphetamine SA. The HR animals showed a lower affinity of type I receptors in the hippocampus (P<0.01) and amygdala (P<0.05), and a reduced affinity of type II receptors only in the hippocampus (P<0.05). The receptors in hippocampus were not different in the two groups. These data suggest that a reduced affinity of corticosteroid receptors in hippocampus and amygdala may be responsible for the higher locomotor response to stress observed in the HR animals. Thus, it is suggested that pharmacological manipulations of corticosteroid receptors may open new therapeutic strategies for compulsive drug use.

494.6


Intra-accumbens d-amphetamine enhances the behavioral control of reward-related stimuli (or conditioned reinforcers) by dopaminergic mechanisms. This study examines the contributions of different limb afferents to the ventral striatum in the amphetamine-induced potentiation of conditioned reinforcement. Subjects were trained to associate a light-noise stimulus (the CR) with reward and then tested in extinction for the acquisition of a new response, maintained by this conditioned reinforcer. Exci-toxotic lesions of either the basolateral amygdala or the ventral subiculum suppressed responding maintained by the conditioned reinforcer, with the greatest effect at the higher doses of intra-accumbens d-amphetamine. Similar results were found following excitotoxic lesions of the ventral striatum itself or following intra-accumbens infusions of low doses of NMDA receptor or quisqualate/AMPA receptor agonists and antagonists. Control experiments assessed the effects of these treatments on sucrose consumption and locomotor activity. The results suggest that the behavioral control of reward-related stimuli is mediated by limb afferents and their interaction with dopaminergic mechanisms in the ventral striatum.

We have previously shown that conditioned tolerance develops to some of the behavioral and endocrine effects of nicotine and to amphetamine (AMPH), as well as to a number of other drugs including morphine, in mice. Two studies that support the feasibility of this idea in male rats. In the first, a single sc injection of 0.75 mg/kg of CORT prevented the analgesic response to sc 0.68 mg/kg of nicotine base (free base) given 5 min later, when compared to untreated controls. In a second study, rats were given AMPH (1 mg/kg) or distilled water) or no injection 10 min before nicotine (75 mg/kg) and tested for nicotine analgesia every other day for 10 days. Significant tolerance developed more rapidly (1st vs 3rd injections) in the CORT pretreated rats. In this study it was used as a nicotine agonist. A similar acceleration was produced by the vehicle pretreatment, which included a significant release of endogenous CORT. The conditioned CORT response could contribute to the development of tolerance to some of nicotine’s effects.

**References:**

494.17

RE-EXAMINATION OF PENTYLENETHETRAZOL (PTZ)-LIKE EFFECT OF NICOTINE (NIC) AND ITS WITHDRAWAL.


Two groups of rats were trained in a 2-lever choice task with nicotine to discriminate the anxiogenic drug PTZ (12 mg/kg or 20 mg/kg) from saline. NIC substituted fully for PTZ and this effect depended upon both dose of NIC and training dose of PTZ. Tolerance to the subjective effect of NIC was evident during chronic infusion of NIC via osmotic pump for 7 days at 0, 10, 20 and 30 mg/kg/d. Chronic NIC dose-dependently reduced lever-pressing, food consumption, weight, and defecation. The magnitude of the weak PTZ-like stimulus previously found during withdrawal (Harris et al., Psychopharmacol. 80:35, 1985) did not depend on dose of chronic NIC nor on training dose of PTZ. These results indicate that NIC-withdrawal is not detected in a systematic manner by the PTZ discrimination. In contrast, the direct PTZ-like effect of NIC is both robust and systematic, suggesting that the acute anxiogenic effect of NIC may be mediated by a mechanism common to PTZ, while the withdrawal symptom "anxiety" is not.

(Supported by AOA 89-07-301)

494.18

PEMPIDINE-INDUCED ANTAGONISM OF PHYSIOLOGICAL, AND BEHAVIORAL RESPONSES TO NICOTINE. W. Guan, R. Bohlke, M.J. Marks, D.G. Collins, Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309

Previous studies have demonstrated that pempidine both competitively and noncompetitively antagonizes central actions of nicotine. The purpose of the present study was to determine if genetic factors influence the sensitivities of mice strains to pempidine blockade of the effects of nicotine in vivo. Both time course and dose response relationships for pempidine antagonism of effects of nicotine were determined. C57BL and DBA mice were pretreated with saline or pempidine (0.5-6.0 mg/kg, ip) at various time points prior to nicotine administration (1.5 mg/kg, ip). The responses to nicotine were measured using a battery of physiological and behavioral tests that includes alterations in respiratory rate, acoustic startle response, Y-maze activity, heart rate and body temperature. Dose response experiments (15 min postinjection) indicated maternal inhibition at a pempidine dose of 1.0 mg/kg. Possible toxicological actions were seen at higher doses. The 1.0 mg/kg dose of pempidine was used to determine a time course for nicotine antagonism; maximal antagonism was seen 20 min following pempidine injection. Pempidine antagonism of nicotine's effects persisted for at least 90 min following acute treatment. Strain differences in sensitivity to pempidine-induced antagonism of nicotine's actions were defined. These results indicate that pempidine is very similar in potency to the classical nicotine antagonist, mecamylamine, and that it is a useful agent for determining the function of brain nicotine receptors. Supported by DA-00116 and DA-03194.

495.2


Vitamin A, in the proper amount and correct time, is crucial for embryonic development but if administered in excess, is a potential teratogen. To test the effects of vitamin A on secondary neurulation and canalization process, pregnant C-57 BL/62 mice were given IF injections of 500 IU (200 μg) vitamin A (retinyl acetate) on gestation day 8.5 and 9.0 (during the time of closure of the posterior neuropores). On successive days, embryos were examined for defects and cultured upon both dorsal and ventral surfaces with light and fluorescence microscopy using a battery of FITC-Lectin and low light video microscopy. The majority of vitamin A embryos were abnormal, with absent tails (18%), kinked/short tails (64%) or caudal neural tube defects (2%). Light microscopy of tail-less embryos revealed alterations in the deposition of the ECM of the neural tube, delays in expression of sialic acid-containing glycoconjugates on the apical neuropil and complex abnormalities. The presence of multiple morphological defects with morphological features of neural tubes. These results suggest that excess vitamin A (or its active metabolites) altered the pre-set developmental timetable responsible for secondary neurulation.

(Supported in part by Kiwanis.)

495.1

ABNORMAL GANGLIOSIDE GD3 SYNTHESIS IN MUTANT MOUSE EMBRYOS. S. Wachtel, N. and Neiderv A., Boston College, Biology Dept., Ossemed., New York, NY 11568

Gangliosides are a family of sialic acid-containing glycolipids that are proposed to function in neural cell-cell interaction and adhesion. Although present in most cells and tissues, ganglioside concentration is greatest in neurons of the CNS. It is generally agreed that in the CNS, ganglioside composition is regulated through two main metabolic pathways. The "a" pathway (comprising gangliosides GM3, GM2, GM1, and GD1a) and the "b" pathway (comprising GL3, GD3, GD2, and GD1b). We previously found significant ganglioside abnormalities in E11 and E12 [μmol/mg] mutant mouse embryos. The "a" pathway is part of the T-locus complex on chromosome 17. These mutants express linker protein deficiency in the developing neural tube. The mutant ganglioside abnormalities involve reductions in "a" pathway gangliosides (especially GD1a) and elevations in "b" pathway gangliosides. We proposed that these abnormalities could arise from a biosynthetic defect in the "b" pathway. To test this hypothesis we examined the [14C]-sialic acid activity of subfractionation of SAT-2 (EC2.4.99.8) in the normal (+/+ and +/tw1) and tw1/tw1 mutant embryos at E-12. This enzyme catalyzes the initial step for the a pathway gangliosides. The enzyme assay was conducted for 4 hours at 10°C and contained (in μM): substrate (GM1Ac, 50 μM), detergent (CF-54, 55 μg), donor (CMF-34-Ac, 25 μM) cadaverine-HCl buffer (pH 6.43), 10 μM containing 1 μM MnCl2 and P2 embryo protein (50-100 μg). The labeled ganglioside products were separated using HPTLC and quantitated using liquid scintillation. The relative specific activity of SAT-2 (expressed as pmol [14C]-sialic acid/h) was significantly lower in the tw1 embryos (227 ± 7, n=3) than in the +/+ (536 ± 24, n=6) or +/tw1 (618 ± 33, n=6) embryos. The absence of an activity in the a pathway enzymes suggests that the an anatomic process that may be a postoxic effect of the mutation. (Supported by NIH grant 24826)

DEVELOPMENTAL DISORDERS OF THE NERVOUS SYSTEM II

The differentiation process of the outflow mechanisms of cerebrospinal fluid (CSF) depends on remodeling of the primitive ventricular system. To examine the time at which the outlets of the IV ventricle become functional, embryonic C57 BL/6J mice (gestation days 13, 14, 15, and 16) were investigated: 1) injection of a CSF marker 125I-BNP into the lateral or mesencephalic ventricles, followed by fixation and scanning electron microscopy. The results of the Prussian Blue reaction product indicate that the outlets of the IV ventricle are functional by gestation day 15, and SEM revealed that a ciliated slit-like opening appeared in the lateral recesses of the IV ventricle. Studies are in progress to apply these approaches to determine whether the IV ventricle outlets and arachnoid development occur normally in genetic models of hydrocephalus (by 3/3 by 3) and the Chilari II malformation (ddp/ddp) in mice. (Supported in part by Elvins.)

DIFFERENTIATION PROCESS OF IV VENTRICLE OUTLETS IN MICE. S. M. Sokol, P. Macario*, and T. H. Beautoymou. of those responsible for procedural planning and execution.

In addition, providing new data on the incidence and nature of developmental dyscalculia, our studies highlight the utility of a cognitive neuropsychological approach to the study of developmental impairments.
A DOUBLE BLIND STUDY OF FOLINIC ACID (LEUCOVORIN)

While formal measures were negative, 50% of the subjects showed side effects for the duration of the study.


FOR THE TREATMENT OF FRAGILE X SYNDROME. W. J. Parvalbumin and calbindin immunoreactive (P-IR and C-IR) cells and terminals were examined in hippocampal tissue removed from patients with MTS in terms of age at onset, diagnosis had been confirmed by karyotype analysis.

The differential distribution of both calcium binding proteins was found in the various cytoarchitectonic regions. The highest density of P-IR terminals with specially high density in the superficial sublayer in CA1. In the dentate gyrus, P-IR neurons were present in the molecular layer and; parent questionnaires. There were no significant differences between placebo and Leucovorin in the behavior of subjects evaluated on the Vineland Adaptive Behavior Scales; Peabody Picture Vocabulary Test; ACTERS, and Leucovorin in the behavior of subjects.

There were no significant differences in blood parameters or side effects for the duration of the study. While formal measures were negative, 50% of the parents reported improvement in their sons.

AGE-RELATED RESPONSES TO OXYGEN DEFICIENCY IN PRETERM AND FULLTERM NEONATAL RATS. M. Dyer and G. R. Allali. Developmental Neurophysiology, St. Louis, MO, 93110

Perinatal oxygen deficiency is associated with neurodevelopmental abnormalities. Behavioral response of pre- and fullterm neonatal rats to acute oxygen deficiency were investigated. Oxygen deficiency was produced by placing fullterm (P0 vaginal delivery) and preterm (E20, E21) Cesarean section delivery rats into hypoxic chambers until they reached a criterion of 3 successive gaspse separated by at least 15 sec each. Control rats were placed in an analogous chamber. Pups were resuscitated by brushing. The procedure was repeated for E21 and P0 rats. Time to reach gasping criterion and survival through 24 hrs postnatal were studied.

Results showed age-related differences in response to oxygen deficiency. Younger rats took significantly more time to reach the criterion. Pups born at less than 4 grams body weight showed lower survival rates.

Neuropathology and transmitter changes due to oxygen deficiency are under study. (Conducted under NIH Guide for Care and Use of Laboratory Animals)
ENHANCED PAIRED-PULSE INHIBITION IN HUMAN EPILEPTOGENIC AMYGDALA AND HIPPOCAMPAL PATHWAYS. S. U. Khan, C. L. Wilson, G. J. Kozlowski and M. E. Levesque. Department of Anatomy and Cell Biology, Department of Neurology, Division of Neurosurgical and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Both during the normal model of epilepsy and deep recordings of seizures onsets in human temporal lobe epilepsy implicated the amygdala nucleus in epileptogenesis. (As an example, see: Neuron, 1989, 15:210) demonstrated greater paired pulse suppression of stimulation evoked population EPSPs in the epileptic hippocampal formation (HF) than in the non-epileptic HF. They attributed this suppression to enhanced GABAergic inhibition produced by principal neuron loss in the hippocampus. Since amygdala and HF have multiple connections, we decided to examine the excitability of these pathways.

For this purpose, we employed paired stimuli in a study of 13 medically intractable complex partial epilepsy patients with deep electrodes implanted in the amygdala and the HF for diagnostic monitoring prior to surgery. Conditioning and test stimuli were delivered to five specific HF sites while recording evoked field potentials from microelectrodes in the amygdala. Stimulation was also delivered to the amygdala during recording from microelectrodes in the HF sites. Paired-pulses were delivered at inter-stimulus intervals of 20 to 1600 msec, and response depression or facilitation was measured by dividing the test by conditioning evoked response slope. The epileptic sites were defined as those on the side of all recorded seizure onsets and the non-epileptic sites as those on the side of no seizure onsets.

The slope of evoked field potentials was significantly suppressed on the epileptogenic side in comparison to the slope on the non-epileptogenic side (F = 9.07, df = 1, p < .005), at interstimulus intervals of 20 to 200 msec. These results indicate interictal inhibition is enhanced in the epileptogenic amygdala, a functional difference consistent with that observed in the non-epileptogenic HF. Supported by NIH grant NS023070.


In TLE, we examined the functional significance of changes in mossy fiber termination, we examined the relationships between Timm-sulfide staining and evoked field responses to orthodromic and antidromic stimuli in in vitro brain slices from the dentate gyri of epileptic patients who underwent temporal lobe resective surgery. We used the same population of patients for these measures to account for variation in their clinical histories. The following physiological parameters were significantly correlated with the intensity of Timm-silver staining in the molecular layer or hilus: 1) early and late inhibition as measured by paired pulse stimulation; 2) change in the orthodromic response during paired-pulse stimulation; and 3) area under the orthodromic waveform. These results are consistent with the possibility that reorganization of mossy fiber terminals from the hilus into the molecular layer may underlie the reordered physiological parameters. Supported by NIH Grant NS-23070 to LMM.

ULTRASTRUCTURAL ORGANIZATION OF SOMATOSTATIN INTERNEURONS IN THE FASCI DENTATA OF HIPPOCAMPI FROM PATIENTS WITH EPILEPSY. M. F. Philips*, C. Pappas*, D. D. Spencer and N. C. de Lanerolle. Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510

Hippocampi surgically removed from patients with medically intractable Temporal Lobe Epilepsy (TLE) can be classified into two groups. Those from subjects with an extra-hippocampal temporal lobe lesion (TLE) have a relatively higher component of hippocampus in contrast to those who have no identifiable lesion (CTLE) [de Lanerolle et al., Brain Res., 495: 387-395, 1989]. Somatostatin-like immunoreactivity (SLI) in the fascia dentata (FD) in TLE was localized in interneuron-like cell bodies in the hilus. Dendrites and axonal processes of these somata ramify in the hilus and some extend through the granule cell layer into the molecular layer (ML). The density of fibers in the inner ML is low compared to the outer ML. In CTLE, SLI somata in the hilus are reduced, but there is an increase in immunoreactive fibers throughout the width of the ML. The participation of SLI elements in the synaptic organization of the FD was studied by an electron microscopic analysis of SLI elements. In CTLE immunostaining for SLI was located in the hilar and inner molecular layer. SLI dendrites were often the center of simple to complex glomeruli with several unlabeled, mostly round vesicles containing terminals. SLI myelinated axons and terminals were commonly seen only in the outer molecular layer and the hilus. The labeled terminals usually contained round vesicles. One main difference in CTLE was the presence of labeled terminals in all regions of the ML. (Supported by NS 27061)

INTERACTION OF ORTHODROMIC AND ANTIDROMIC STIMULI IN THE DENTATE GYRUS OF EPILEPTIC PATIENTS IN VITRO. K. Uyeda, M.J. O'Connor and L.M. Maslava. Departments of Neurology, Radiology, and Cell Biology, Division of Neurosurgical and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Neuronal loss and mossy fiber reorganization occur during the course of epilepsy. Alterations in circuitry are thought to be associated with functional changes. In order to elucidate changes in function and circuitry, field responses of granule cells to paired pulse stimuli were examined in the dentate gyrus of hippocampal slices from temporal lobe epilepsy (TLE) patients and normal rats. Paired pulse stimuli (orthodromic pair, antidromic pair and combinations of orthodromic and antidromic pairs) were tested at various interstimulus intervals (ISI) via electrodes placed in the molecular layer and in the hilus. In slices of TLE patients, the first stimulus (either orthodromic or antidromic) inhibited the second antidromically evoked population spike at short ISIs. This inhibition was stronger in the slices which showed hyperexcitability of granule cells. The inhibition was enhanced by bicuculline, and blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 2-amino-5-phosphonovaleric acid (APV) partially reduced the inhibition. Neither the inhibition nor its enhancement by bicuculline was significant in rat slices. Orthodromic paired pulse inhibition was well preserved in some slices that showed antidromic paired pulse inhibition in TLE patients. These results suggest that mossy fibers make functional synapses with inhibitory interneurons (possibly non-GABAergic), in addition to excitatory synapses with granule cells. Presence of the new inhibitory inputs may precede the progressive decrease or loss in orthodromic paired pulse inhibition observed in TLE patients. Supported by NIH grant NS23077 to LMM.

DYNAMIC ANALYSIS OF CARDIAC RATE DURING COMPLEX PARTIAL SEIZURES. B. C. Fostinger, S. Garai, M. E. Levesque and R. M. Harper. Dept. of Anatomy and Cell Biology, Div. of Neurology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Heart rate has been shown to change dramatically and systematically during epileptic seizures. Such changes may involve mesial temporal structures such as hippocampus and amygdala, which have been shown to play a role in autonomic control as well as epileptogenesis. Heart rate patterns can thus serve as identifying features of seizure discharge. We examined perievent heart beat-by-heartbeat interval records of patients undergoing continuous seizure monitoring. Poincaré plots (RRi+1 vs. RRi) of intervals were calculated, and these plots indicated marked dynamic changes in interval interval variation during a seizure. To quantify variability at low (0-0.5 Hz), mid (0.5-15 Hz) and high (> .15 Hz) frequencies, cardiac interval records were interpolated to provide a linear time series, and FFTs were calculated on successive 16.38 second periods around each seizure. We derived two measures from each period: broad-band power (a measure of total variability), and the ratio of low to high frequency variation (an inferential measure of the relative contributions of sympathetic and parasympathetic sources). Results indicate that preictal cardiac variability is state-dependent, moderate and variable in frequency dominance. At seizure onset a large increase in broad-band power and in the low/high ratio occurs, suggesting a dramatic increase in sympathetic input. Low frequency power decreases over the course of the seizure, while high frequencies increase, probably reflecting significant co-activation of sympathetic and parasympathetic sources. These techniques provide quantification of degree and type of autonomic activation associated with seizures. An important factor in seizure characterization and cardiovascular risk assessment. The computationally simple Poincaré plots provide a qualitative display of the dynamic characteristics of the variability which could be provided in real time. Supported by NS03808.


Using [3H]cytidine as a precursor, it has been demonstrated that PI turnover in tissue slices can be stimulated by a variety of compounds ranging in the accumulation of membrane bound [3H]cytidine diphosphate diacylglycerol ([3H]CDP-DAG)(Science 249: 802 (1990)). The non-specific levels of non-membrane bound precursor and radiolabeled nuclei acids were removed by washing, and the autoradiographic images generated from these labeled sections reflects the degree of PI turnover in fairly discrete neuroanatomical regions. Living human brain tissue was obtained from patients undergoing surgery for medically intractable temporal lobe epilepsy. Slices were prepared from either hippocampus or neocortex and preincubated with [3H]cytidine. The slice was then incubated with LICI +/- carbachol. The slice was cut into 20um sections before being washed and exposed to the autoradiographic images generated from these labeled sections reflects the degree of PI turnover in fairly discrete neuroanatomical regions. Living human brain tissue was obtained from patients undergoing surgery for medically intractable temporal lobe epilepsy. Slices were prepared from either hippocampus or neocortex and preincubated with [3H]cytidine. The slice was then incubated with LICI +/- carbachol. The slice was cut into 20um sections before being washed and exposed to the autoradiographic images generated from these labeled sections reflects the degree of PI turnover in fairly discrete neuroanatomical regions.

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EPILEPSY: EPIDEMIOLOGY AND ANIMAL MODELS

496.11
STIMULATION EVOKED POPULATION SPIKES IN HUMAN EPILEPTOGENIC HIPPOCAMPAL FORMATION SHOW ENHANCED INHIBITION. C.L. Wilson, S.U. Khan, H.F. Hat, and M.F. Levesque, Department of Anatomy and Cell Biology, Dept. of Neurology, Div. of Neurological Surgery and Brain Research Inst., UCLA School of Medicine, Los Angeles, CA 90024.

496.12

496.13
THE FREEZE LESION EPILEPSY MODEL ADAPTED TO RATS. W.D. Knowles and W.R. Barrett, Epilepsy Basic Research Program, Cleveland Clinic Foundation Research Institute, Cleveland, OH 44195.

496.14
A SYSTEM FOR PRODUCING BENZODIAZEPINE TOLERANCE IN MICE USING OSMOTIC PUMPS. C.D. Torchin*, I.M. Kapetanovic, and H.L. Kupperberg*. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

497.1
THE GABA UPTAKE INHIBITOR TIAGABINE INCREASES EXTRACELLULAR BRAIN LEVELS OF GABA IN AWAKE RATS. A. Flink-Jensen, Medical Br., Galveston TX, 77550.

497.2
ANTICONVULSIVE ACTIVITY OF ANTAGONISTS AT THE GLYCINE MODULATORY SITE ON THE NMDA RECEPTOR COMPLEX. Alfred C. Nichols and E. Lemon Yielding*, Dep. of Pharmacology, Univ. of Texas Medical Br., Galveston TX, 77550.

497.1
Anticonvulsant activity of analogs of tiagabine, a GABA uptake inhibitor. Tolerance to the anticonvulsant effects of benzodiazepines limits their use in epilepsy therapy. Animal models demonstrating tolerance have been developed, but they require repetitive injections over several days or usil saline capsules which must be made for each drug and do not provide a consistent infusion rate. Alzet® 2001 osmotic minipumps deliver at a constant rate (1 μl/hr) and dosage can be easily adjusted. Various studies in rats and mice demonstrate that chronic infusion produces long-term anticonvulsant effects.

497.2
Anticonvulsant activity of tiagabine (T), tiagabine hydrochloride (HT), and tiagabine citrate (CT) in the maximal electroshock seizure (MES) and pentylenetetrazol (PTZ) tests in mice. Tiagabine (T) and the structurally related, non-competitive GABA uptake inhibitor zonisamide (Z) were compared with tiagabine citrate (CT) and tiagabine hydrochloride (HT). In the MES test, all drugs were administered by constant-rate osmotic minipumps. This model could be useful to screen putative anticonvulsant drugs for development of tolerance.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
497.3 VALPROIC ACID ENHANCES THE IPSP IN THE RAT AMYGDALA. L.-M. Tian and K.-A. Alkadhi. Department of Pharmacology, University of Houston, Houston, TX 77204-5515.

Valproic acid (VPA) has been widely used clinically as an antiepileptic drug, but the mechanism of its action has not been fully established. In this study, we have examined the effect of VPA on the amygdala neurons in vitro using intracellular recording techniques. Amygdala slices (300 μm) were prepared from male Sprague-Dawley rats and bathed in artificial cerebrospinal fluid (ACSF).

The resting membrane potential and input resistance of the amygdala basolateral nucleus neurons were -65 to -70 mV and 40 to 80 MΩ, respectively. Addition of VPA (30-100 μM) to the superfuse caused hyperpolarization of -5.5 mV ( ± 4.3 mV) and increased input resistance of amygdala neurons. Stimulation of the amygdalo-fugal pathway evoked inhibitory postsynaptic potentials (IPSP). This IPSP could be eliminated by hyperpolarizing the membrane to -75 mV or by addition of the GABA(A) antagonist bicuculline (10 μM) in the superfuse. The IPSP amplitude increased 1.3-fold after 10 minutes of superfusion with VPA (100 μM) when the membrane potential was artificially held at the resting level.

When slices were bathed in bicuculline (10 μM) for 5 min, an epileptiform activity was observed upon subthreshold stimulation of the amygdalo-fugal pathway. The bicuculline-induced epileptiform response was completely abolished 15 minutes after addition of VPA (100 μM). These preliminary results suggest that VPA changes the membrane properties of the amygdala basolateral nucleus neurons probably by modifying the GABA(A) receptor function.


It has been known that seizure activity is associated with increases in the proto-oncogene c-fos mRNA and c-fos protein in brain. The present study was undertaken to determine the presence of an anticonvulsant kappa opioid drug. PD117302 (Tortella et al, Life Sci., 46, PL 1, 1990), and the NMDA antagonist MK801, would block seizure-induced c-fos mRNA in rat brain. c-fos mRNA levels, measured by Northern blot analysis, were greatly increased in a time-dependent manner following maximal electroshock (MES). Increased levels were measured within 15 min after MES, reaching a maximum in 30 min and declining to control levels within 2 hr post-convulsion. A maximal stimulation in c-fos mRNA of 10-15 fold was observed in the cerebellum, followed by hippocampus (5-7 fold) and neocortex (4-6 fold). Administration of anticonvulsant doses of PD117302 (16 and 32 mg/kg, s.c.) and MK801 (0.2, 1.0 and 2.0 mg/kg, s.c.) alone produced no significant changes in c-fos mRNA levels in these three brain areas. However, both drugs (PD117302, 32 mg/kg; MK801, 0.2 mg/kg) administered 30 min prior to MES significantly blocked c-fos mRNA induction with the greatest effect observed in the cerebellum and a minimum effect in the neocortex. Seizure-induced increases in c-fos mRNA levels and its partial blockade by anticonvulsant drugs suggest a possible role of c-fos in the mechanism of action of these drugs.


CARSAMIPHEN is a potent anticonvulsant agent which is a more effective antiseizure than atropine and also potently blocks MES-induced seizures in mice and rats. The anticonvulsant mechanism of carisamphen has been hypothesized to be due to high affinity binding to dextromethorphan (DM)-labelled σ recognition sites in brain. We initially examined four properly chosen para-substituted carisamphen analogs to determine the influence of aromatic substituent parameters (i.e., σ, n) on σ receptor binding and also to determine if there was a correlation of σ binding affinity with anticonvulsant activity. Some of the analogs potently inhibited σ binding but were devoid of anticonvulsant activity. Of the compounds evaluated in MES only the 4-amino derivative showed anticonvulsant activity (ED50 = 3 mg/kg), although its σ binding assays its affinity was less than that of carisamphen. Comparable structure-activity data has previously been reported for 3-substituted DM analogs. In both series, while no apparent correlation exists between σ affinity with anticonvulsant activity, this information may reveal the common modes of binding of carisamphen and DM analogs to the σ recognition site.

497.6 CROSS TOLERANCE BETWEEN CARBAMAZEPINE AND VALPROATE IN AN AMYGDALA KINDLED SEIZURE PARADIGM. S.R. Weiss, R. Lewis, E. Sohn, A. Berger, R.M. Post. Biological Psychiatry Branch, NIH, Bethesda, MD 20892.

We have previously demonstrated the importance of the peripheral benzodiazepine receptor (P-BzR) in the anticonvulsant effects of amygdala kindled seizures. In order to evaluate potential common mechanisms of action, we evaluated cross-tolerance between CBZ and other anticonvulsant drugs. When tolerance to CBZ was induced, animals showed a lack of anticonvulsant response to PK-11195 (an antagonist at the P-BzR) and sodium valproate (VPA), but not to diazepam (DZP). Since the P-BzR is found in greatest concentrations in the olfactory bulb in rats, and VPA has recently been shown to be distributed almost exclusively in this area following in vivo administration (Hoepfner, 1989), we examined what effect lesions of the olfactory bulb would have on anticonvulsant responsiveness. Rats with lesions of the olfactory bulb developed amygdala kindled seizures at rates equivalent to rats with sham lesions, and they showed no cross-tolerance to CBZ, VPA, and DZP.

Our results suggest that while the P-BzR is important for the anticonvulsant efficacy of carbamazepine, receptors in the olfactory bulb are not critical for this effect. Similarly, although radioabeled VPA is highly localized to the olfactory bulb, this structure is not necessary for the anticonvulsant effects of VPA. Potential common mechanisms of anticonvulsant action of CBZ and VPA are suggested by our findings of cross tolerance and require further investigation.

497.7 LAMOTRIGINE AND PHENYTOIN INTERACTIONS ON IONIC CURRENTS PRESENT IN N4TG1 AND GH3 CLONAL CELLS. D.G. Lange and C.M. Hanks. Division of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Lamotrigine (LTG) is a novel anticonvulsant with a unique chemical profile; it has a pharmacological profile similar to phenytoin (PHT). Epilepsia 27:463-469, 1986). LTG, like PHT, inhibits veratrine-induced glutamate release from rat brain cortical slices, pretreated with iodoacetate (IA) in the bath solution, to result in a decrease in the glutamate release of 40-45%. It has been known that seizure activity is associated with increases in the proto-oncogene c-fos mRNA and c-fos protein in brain. The present study was undertaken to determine if the novel anticonvulsant LTG blocks the increase in the glutamate release of 40-45% produced by PHT or by IA pretreatment. Pretreatment with memantine (MEM, 18 mg/kg, sc) + PHT or IA had no effect on the LTG-induced decrease in glutamate release. LTG (100 μg/kg, sc), produced limbic seizures in rats. LTG also caused a significant increase in the glutamate release of 57% when given in combination with PHT or IA. These results suggest that the partial antagonism of PHT by LTG may be due to a decrease in the glutamate release induced by PHT or IA. The observations also suggest that LTG may be useful in the treatment of seizures associated with increases in glutamate release.


A single sublethal dose of the nerve agent, SOMAN (150 μg/kg, sc), produced limbic seizures. Pretreatment with memantine (MEM, 18 mg/kg, sc) + atropine sulfate (ATS, 16 mg/kg, sc), but not ATS, prevented seizures. MEM, but not ATS attenuated seizure activity in progress. MEM+ATS or ATS had no effect on acetylcholinesterase (ACHE) activity in crude brain homogenates. Pretreatment with MEM reduced inhibition of ACH activity by SOMAN, but not edrophonium (cathionic site) or decamethonium (peripheral site inhibitor), suggesting action at a different modulatory site. MEM limited action potential firing frequency, and both MEM and ATS blocked ACh responses recorded intracellularly from cultured neurons. Thus, protection against SOMAN seizures by MEM+ATS resulted from a combination of biochemical and pharmacological effects.
497.9 VOLTAGE-SENSITIVE CALCIUM CHANNELS (VSCCs) OF MOUSE CORTICAL ASTROCYTES ARE ATTENUATED BY PROTOTYPE ANTICONVULSANTS NANTS S. Cai, J. Edwards1, D.M. Woodbury2 and H.S. White1. 1Dept. of Pharmacology & 2Dept. of Physiology, University of Utah, Salt Lake City, Utah 84108

 Previous investigations in this laboratory have demonstrated that primary cultures of mouse cortical astrocytes express VSCCs which are effectively blocked by the benzodiazepine and the N-type channel blocker 1,5-dihydropyridine. Since a number of anticonvulsants have been shown to block VSCCs of neurons, the present study was initiated in order to assess the effect of VSCCs in astrocytes using the fluorescent probe indo-1. Astrocytes grown on 25 mm cover slips and maintained in tissue culture for 20-31 days were "loaded" with indo-1 (5 μM) for 20 min. and fluorescence measured according to the method of Peeters et al. Depolarization-activated astrocytes with elevated K+ (55 mM) resulted in a significant increase in the free [Ca2+]i concentration as estimated by an increase in the 410/480nm fluorescence intensity ratio (Grynkiewicz et al. J.Biol Chem 260:3440-3450, 1985). Pretreatment for 5 min. with therapeutic concentrations of phenobarbital (10 μM); valporate (1 μM); diazepam (1 μM); ethosuximide (100 μM); phenytoin (3 μM) and lanzarine (10μM) attenuated the peak KCl-induced Ca2+ transients by 84, 82, 81, 78, 71, and 60%, respectively. Whether these drugs are blocking Ca2+ influx through a direct interaction with the channel (L or N) or through an effect on second messenger systems is currently under investigation. In summary, the present results suggest that the VSCCs of astrocytes are amenable to pharmacological manipulation by anticonvulsant compounds. Supported by a grant from the NINDS of the NIH (2PO1-NS-22200).


Calcium channel antagonists (CAs) are effective anticonvulsants in several models of epilepsy. The present study determined if nifedipine (NIF) and verapamil (VER) would protect Sprague-Dawley rats from convulsions induced by bicuculline (BIC) or picrotoxin (PIC) or bicuculline (BIC). At 15 or 30 days of age, pups were pretreated i.p., with either phenobarbital (15 mg/kg, 45 min.), NIF (20 mg/kg, 30 min) or VER (20 mg/kg, 10 min) prior to s.c. injection of PTZ (50 mg/kg), PIC (3.5 mg/kg) or BIC (2.7 mg/kg). Controls received 0.1 ml/100 body weight DMSO. Three indices of epileptiform behavior were measured for 30 minutes. At PND 15, NIF reduced head/thrasm movements (HLM) and body rotation (BR) induced by BIC, PIC and PIC, in contrast, NIF pretreatment enhanced seizure-like activity (SLA) induced by PIC and BIC but not PTZ. VER reduced HLM movements induced by all 3 chemical convulsants and BR following BIC and PIC. PIC induced SLA was enhanced by VER pretreatment. At PND 30, NIF pretreatment enhanced SLA of PTZ and PIC, it was ineffective in reducing other indices. VER had no effect on PTZ, BIC or PIC convulsions. The results suggest that in developing rats CAs exhibited limited anticonvulsant efficacy and may exacerbate some chemically-induced seizures. (Supported by PHS Grant #5R01-NS02725).

497.11 A ROLE FOR SEROTONIN IN THE ANTICONVULSANT EFFECT OF ANTIPEPTIDESININE IN GENETICALLY EPILEPSY-PRONE RATS, Q.S. Yeh, E.C. Jada and J.W. Dals, Department of Biological Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61655.

 Previous reports from our laboratories demonstrate that anti-peptidespine (3,4-methylene dioxyamphetamine, AE), which is clinically used as an antidepressant in China, is an effective anticonvulsant in genetically epilepsy-prone rats (GEPRs) and that the anticonvulsant effect of AE in moderate seizure GEPRs (GEPR-3a) was mediated at least in part by activation of CNS serotonergic neurons (FASEB. J. Abs. 3: A292, 1989; Juntendo Fdn.). Therefore, in order to further test this hypothesis linking the anti-peptidespine action of AE to CNS serotoninergic neurons, enhanced serotonin release, we evaluated the effects on diazoxide sensitive i.p. of AE in severe seizure GEPRs (GEPR-9a) and of i.v. AE in GEPR-3a. Under anesthesia, gently restrained rats were stereotactically injected over hippocampus of both GEPR-9a and GEPR-3a. To inject i.v. AE without stress, the GEPR-3a were also implanted with a jugular catheter. After recovery from surgery, dialysis probes were inserted into the guides of the conscious and unrestrained animals and the hippocampus was dialyzed with artificial CSF. Either (32 μg/kg i.p. in GEPR-9a; 40 μg/kg i.v. in GEPR-3a) or vehicle was administered after establishing basal release and dialysis was continued for 6 hours. Significant increases in dialysate serotonin (approximately 200-250% over the basal values) were seen after the injection of AE in both GEPR-9a and GEPR-3a. The peak increases in serotonin produced by i.p. AE corresponded temporally with the time of peak anticonvulsant effect for this drug. These results confirm our earlier observation and are consistent with a role for serotonin in the anticonvulsant effect of AE in GEPRs. (Supported in part by a grant from Tsunuma Juntendo Fdn.)


Earlier investigations in our laboratory have shown lidocaine to be an effective agent in the cessation of ongoing seizures when microinfused into an experimentally-produced focus. While the effectiveness of this technique has been verified behaviorally (1), quantification of the ECoG has not been performed to date. Although behavioral changes in are associated with abnormal discharges which have spread to the motor cortex, ECoG changes provide information about seizure activity occurring in other cortical and subcortical areas as well as provide information about abnormalities in expression of focus seizures. The present study used both behavioral and electroencephalographic measures to quantitatively evaluate the effectiveness of the lidocaine technique.

Ten Long-Evans rats were used for Experiment 1, rats received a pentobarbital injection (15 mg/kg, 30 min) and then bilateral carotid cannulas were placed over temporal areas (Tempests (AT), and with cortical electrodes overlaying the frontal and parietal cortices. Seizures were induced via a unilateral microinfusion of 0.5 μl of bicuculline (200 μg/ml) into the right MCA at 15 min after micropipette record. Fifteen minutes later, 1 μl of lidocaine or saline was microinfused into the same area. The results showed that lidocaine significantly reduced both the behavioral seizure, as measured on the Racine motor rating scale (2), and the integrated ECoG amplitudes to baseline levels. Saline did not cause a deviation in either measure from seizure levels. These results indicate that lidocaine may prove to be a useful means by which to arrest ongoing focal seizures.


497.13 CLASSIFICATION OF SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS BASED ON RESPONSES TO IONTOPHORETIC GABA AND FLURAZEPAM, B.C. Rosenzweig, H. Zhang and T. Ment, Dept. of Pharmacology, Med. Coll. of Ohio, Toledo OH 43699.

Previous work showed that intragranal GABA agonists and benzodiazepines (BDZs) had differential effects on some experimental seizures (Zhang et al., 1999,1991). It was suggested that SNpr might be differentially based on their responses to GABA and BDZ. Male, Sprague-Dawley rats were anesthetized, and anesthesia maintained by a constant level of chloral hydrate (300 mg/kg). Multi-barrel glass electrode assemblies were used to record spontaneous activity of single SNpr neurons. GABA and flurazepam (FZP), a benzodiazepine, were applied iontophoretically, using several currents for each agent. Recording sites were marked histologically confirmed. As expected, both FZP and GABA, as a function of increasing current, decreased the firing rate of neuronal discharge. The maximum effect obtained with increasing currents of each agent, the neurons could be divided into categories. Most SNpr neurons were inhibited >90% by GABA, but could be inhibited only about 50% by FZP. Other neurons were inhibited >90% by both FZP and GABA. A few neurons were relatively insensitive to FZP and GABA, and a few could be inhibited more by FZP than by GABA. The data suggest that SNpr neurons can be differentiated by their relative sensitivity to FZP and GABA. Histological examination suggested some anatomical segregation according to B2 sensitivity. Supported by DA02194.


The amygdala-kindling model of epilepsy was used to examine contingent tolerance and cross tolerance to the anticonvulsant effects of pentobarbital (15 mg/kg, IP) and ethanol (1.5g/kg, IP) in male Long-Evans rats. In Experiment 1, rats received a pentobarbital injection and a convulsive stimulation once every 48 hr on each of 10 trials. Injection of ethanol was delayed until before or 1 hr after each stimulation. Only rats receiving pentobarbital before each stimulation became tolerant to pentobarbital’s anticonvulsant effect. Cross tolerance to the anticonvulsant effect of ethanol was also found to be greater in the pentobarbital-before-stimulation rats. Experiment 2 assessed the transfer of tolerance from ethanol to pentobarbital. Results mirrored those of Experiment 1: Convulsive stimulation during the ethanol exposure facilitated the development of tolerance to the anticonvulsant effect of ethanol and its transfer to pentobarbital. The development of tolerance and cross tolerance to anticonvulsant drugs effects is facilitated by the administration of convulsive stimulation during periods of drug exposure. This supports the drug-effect theory of tolerance. (MRC and NSERC grants to JPJP)
Reduced Blood Flow in the Temporal Lobes in Alzheimer's Disease

J.I. Ebeling, W.J. Jagust, B.R. Reed*, M.G. Baker, Denny Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Alzheimer's disease (AD) is characterized by reduced cerebral blood flow and neuronal loss especially in parietal and temporal cortices. This has been reported with both single photon emission computed tomography (SPECT) and positron emission tomography (PET). In this study, SPECT was used to study regional cerebral blood flow (rCBF) in 50 mildy and moderately demented AD patients and non-demented control subjects in order to evaluate CBF in the frontal, temporal, parietal, and occipital cortices. The results show that rCBF in temporal cortex is significantly lower than controls for both the mild and moderate AD patients. The correlation between CBF and severity of dementia, as measured by the MMSE, and CBF for the AD patients. Results of the MANOVA showed that rCBF in temporal cortex was significantly (p < 0.0001) lower than controls for both the mild and moderate AD patients, while rCBF in parietal cortex was significantly (p < 0.0001) lower than controls for both the mild and moderate AD patients. These results suggest that temporal cortex is affected earlier and more severely in AD, and that other cortical areas become involved as the disease progresses.

Prolonged MR T2 Times in the Hippocampus and Amygdala Mark the Presence and Severity of Alzheimer's Disease

S. J. Kresh, R. J. Lane, J. L. Butcher, and J. Brayton, Behavioral Neuro-science Program, Department of Psychology, UCLA, Los Angeles, CA 90024.

Magnetic resonance (MR) imaging is a well-accepted visualization tool that produces high quality macroscopic images of body tissues based on the excitation of hydrogen nucleus. By simultaneously processing hydrogen nuclei contribute to an MR image: proton density, T1 relaxation, and T2 relaxation. Thus, the data from which these macroscopic images are constructed arise from molecular properties of the tissue. By quantitatively examining MR signal intensity concerning molecular characteristics of tissues can be deduced. Taking this approach, we measured T2 relaxation times in the left and right hippocampi (HC) and amygdala (AM) of 12 patients with Alzheimer's disease (AD), 9 subjects with multi-intact dementia (MID), 11 elderly normals (EN), and 23 young normals (YN). Using an Instrumentarium Magnaview MRI operating at 0.41 T (SE: 100/130 and 1000/200), T2 values for all AD patients exceeded that of any nondemented individual, regardless of age. Further, HC T2 values were normal for the 9 subjects with MID. Finally, the degree of T2 prolongation was highly correlated with the severity of functional and cognitive impairment of the AD patients. A second study is now underway which preliminary observations indicate that T2 times are also prolonged in the amygdala of AD patients, but not YN, EN, or MID subjects. These results suggest that HC, and possibly amygdala, T2 prolongation may provide a specific anatomical marker by which AD pathology may be characterized and followed in vivo.

Patterns of InterCorrelations of Regional Cerebral Metabolic Rates of Glucose (rCMRglc) in Patients with Dementia of the Alzheimer Type (DAT)


We have tested our hypothesis by asking: do individuals with more years of education have a more advanced stage at the beginning of the dementia process before it is clinically evident? In AD, there is a specific pattern of cerebral blood flow reduction in the temporoparietal area, as measured regional cerebral blood flow, using 15N 2-hydroxylate technique in 3 groups of patients with probable AD matched for severity of dementia, but with varying levels of education (N=36). Although global flow was comparable across the three groups, the temporoparietal perfusion deficit was significantly greater in the group with the highest level of education (p < 0.05). This suggests that AD was more advanced in the higher education group although measures of the clinical severity of the dementia were comparable across groups. We conclude that increased education is associated with a reserve which compensates for the neuropathological changes of AD and delays the onset of its clinical manifestations.

Patterns of InterCorrelations of Regional Cerebral Metabolic Rates of Glucose (rCMRglc) in Patients with Dementia of the Alzheimer Type (DAT)

H. E. Azari, S. J. Rapoport, C. L. Grady, M. B. Schapiro*, and B. Horwitz, Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892.

A correlational analysis of resting rCMRglc data obtained using positron emission tomography (PET) with [18F]Fluoro-D-glucose, examined differences between DAT and control (CTRL) subjects. 22 age- and sex-matched CTRLs and 19 mild/moderate DATs were scanned with a Scanditronix PClO24-7B tomograph. The brain was divided into 65 regions of interest (ROIs). DATs had lower metabolic rates than CTRLs, and a greater variability of metabolic asymmetry. Correlations were performed on normalized (to whole-brain) values. DATs had lower total correlations with CTRLs and showed decreased fronto-parietal, frontal-sensorimotor, fronto-lingual, and sensorimotor-parietal correlations. ROIs involved in the decreased DAT correlations were dorsal left prefrontal, ventral left sensorimotor, right/left inferior parietal, right/left frontal, and entorhinal cortex. The results, continuing and extending a prior correlation analysis using a lower resolution PET scanner and different DAT and CTRL subjects (Horwitz et al., 1987) demonstrate decreased corticocortical interactions in DAT.

Elevated levels of pancreatic thread protein (PTP)-immunoreactivity have been reported in brains of AD patients with Alzheimer's Disease (AD). Antibodies to PTP showed cross-reactive epitopes with a protein in AD brains. Further analysis of this neural thread protein-like immunoreactivity (NTP) revealed a larger protein species (20 KD) both in the brain and cerebrospinal fluid (CSF) as compared to the pancreatic form of the protein (14KD). We have developed an automated microparticle enzyme immunoassay (MEIA) for NTP. The reagents for this assay have been optimized and stabilized. Up to 22 samples can be analyzed within 45 minutes with a sensitivity of 60 picogram/ml. Titration curves of both normal and AD CSF demonstrated a linear relationship as did normal and AD brain extracts. Preliminary results indicate that clinically diagnosed probable AD CSF specimens have much higher NTP levels than those of non-AD CSF.


Alzheimer's disease (AD) is a neurodegenerative disease characterized by a chronically deteriorating course of impaired intellectual function and memory loss. The definitive diagnosis of AD is made by pathological examination of postmortem brain tissue in conjunction with a clinical history of dementia. The antemortem diagnosis of AD, however, is by exclusion. The misdiagnosis rate is about 30%. We have demonstrated in a large population of patients (>400) that ADAP is present in AD brain tissues and not in non-AD. Studies involving the distribution of ADAP in the brain have revealed that ADAP is elevated in the brain regions associated with memory processing/storage. Hence, we expect ADAP in CSF to be a good antemortem marker for AD. The ADAP concentration in CSF is in the femtomole/ml range. Using ADAP extracted from brain tissue as a model, we have developed a chemiluminescent immunoassay (CLI) suitable for measuring ADAP in CSF. Preliminary results indicate that ADAP level is significantly higher in AD CSF specimens as compared to non-AD CSF specimens.


The occurrence of 5-S-cysteinyl-dopamine (5-S-cysteinyl-DA), 5-S-cysteinyl-3,4-dihydroxyphenylalanine (5-S-cysteinyl-DOPA) and 5-S-cysteinyl-3,4-dihydroxyphenylacetic acid (5-S-cysteinyl-DOPAC) in dopaminergic brain areas of several mammalian species has been reported by this laboratory. DA metabolites such as 5-S-cysteinyl-DOPAC and related catechols undergo autoxidation to reactive quinone forms, which then interact with thiol groups as thiocarbonyls. 5-S-cysteinyl-DOPAC has been shown to be autoxidized in vivo by brain tissue extracts. A substantial increase in 5-S-cysteinyl-DA has been found in the striatum following different time periods of monamine treatment (5 mg/kg, i.p.). It was suggested that reserpine's inhibition of vesicle storage caused an increase in autoxidation of the unlabeled cysteine pool of DA. Co-administration of parargyline (75 mg/kg, i.p., 18 h) did not change the elevation in 5-S-cysteinyl-DA levels, although DA available for autoxidation must have been increased. However, following parargyline treatment alone 5-S-cysteinyl-DA levels remained significantly higher than controls. Parargyline treatment does not appear to be lower for the 5-S-cysteinyl adducts than for their parent catechols as indicated in studies on enzyme inhibition as well as in other studies. Following a 1 h of monamine oxidase or tyrosine hydroxylase levels, the levels of DOPAC and DOPA, respectively, decreased significantly whereas no effect was observed for the correlated adduct's levels. Following 18 h of parargyline treatment the 5-S-cysteinyl-DOPAC concentration had decreased drastically, although not as much as the concentration of DOPAC. Alpha-Methyltryptophane treatment for 8 h (250 mg/kg, i.p.) of reserpine pre-treated animals resulted in significantly decreased levels of all three cysteinyl adducts.

499.2 LOCALIZATION OF SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE IN ANATOMICAL AREAS ASSOCIATED WITH NEURODEGENERATIVE DISORDERS. P. Dehmer and S. Schneider. Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430. Dep. of Neurology, Hahnemann University, Philadelphia, PA 19102.

Superoxide dismutase and glutathione peroxidase are two key enzymes which protect cells from destructive oxygen radicals. There is a growing body of evidence which implicates potentially damaging oxygen free radicals in disease-associated oxidative damage. Each of these enzymes has been identified in the human and rodent brain (Soc. for Neuroscience Abst., 1991). In the present study, we focus on areas strongly correlated with two important neurodegenerative disorders. Alzheimer's Disease (hippocampus, nucleus basalis) and Parkinson's Disease (substantia nigra and ventral tegmental area). It has been possible to identify the protective enzymes of interest in melanin-containing cells of the substantia nigra in primates and in cells of the cortex and hippocampus (granule cell layer). The sub-regional localization of these enzymes in these anatomical areas may have important implications for the pathophysiology of these neurodegenerative diseases.

Monoamine oxidases A and B are involved in degradative deamination of biogenic amines, including catecholamine neurotransmitters that are encoded in homologous genes near each other on chromosome Xp11.3. Alleles for the MAOA gene are marked by several RFLPs, as well as a (GT)n repeat (Black et al, NAR, 1991); those for the MAOB gene, by a (CA)n repeat. A strong association has been found in control between levels of MAO-A activity, measured in human skin fibroblasts, and specific alleles for the MAOA gene. Similar predictive alleles are seen for MAO-B activity, measured in platelets, and MAO-B alleles. Individuals inherit widely varying levels of MAO-A and MAO-B activities. These different activity states may predispose individuals to different diseases, like Parkinson's disease, where drug models have implicated MAO in the pathogenic process. We have found a statistically significant difference in the frequency of alleles for MAOA and MAOB in over 60 Parkinson's patients and 60 controls. This finding implicates endogenous levels of MAO-A and MAO-B activity in the disease process, and suggests that allele status for these genes may be an index of susceptibility.

499.4 CALBINDIN-D28K IN THE HUMAN HYPOTHALAMUS: RELATIONSHIP TO TYROSINE HYDROXYLASE (TH) IMMUNE-ACTIVE NEURONS. M.K. Sanghera and D.C. Gerber. Dept. of Psychiatry, UT Southwestern Med. Ctr., Dallas, TX.

Calbindin-D28k (CaBP), a high-affinity calcium-binding protein, is localized within the mitochondrion dopaminergic (DA) neurons in the same regions that are spared in Parkinson's disease (PD) (German et al., Neurosci. Abstr. 16:696, 1990). Hypothalamic DA neurons do not appear to be degenerated in PD (Matzuk & Saper, Ann. Neurol. 18:552, 1985). This present experiment sought to determine if CaBP is localized to specific nuclei of the DA neurons. Immunohistochemical staining, with antibodies against TH (1:1000) and CaBP (1:1000), and computer imaging techniques were used to map the distribution of CaBP and TH-containing cells. CaBP cells were located throughout the hypothalamus, however, often not confined within specific hypothalamic nuclei. The TH-containing cells were found within such nuclei as the arcuate, periventricular and arcuate nuclei. There was no substantial overlap in the distributions of CaBP and TH-containing cells. These data suggest that CaBP does not protect the hypothalamic DA neurons from degeneration in PD. Supported by American Parkinson Disease Association, and AG-08013.
ANTI-NEURONAL ANTIBODIES AND OTHER Markers of Immune System Activation in Parkinson’s Disease. G. Kapatos3, J.B. Peter*2, P.A. Lewitt*1 Dept. of Psychiatry, Wayne State University3, Detroit, MI 48201 and The Clinical Neuroscience Program1, Sinai Hospital, Detroit, MI 48235 and Specialty Labs, Inc.*3, Santa Monica, CA 90404-3900.

Defects of Parkinson’s Disease. E.M. DeMet, C. Reist, University3, Detroit, MI 48201 and The Clinical Neuroscience Program1, Sinai Psychiatry, Univ. California, Irvine, CA 92717 and Long Beach VA unmedicated PD subjects (n = 17), normal controls (n = l3 ), and patients with immune mechanisms in this disorder have not been explored. With CSF from patients with Parkinson’s Disease (PD), though further clues for study sought to determine if similar changes also occur in retina.

Evaluating possible anti-neuronal immunoglobulins: an enzyme-linked immunosorbent assay (ELISA) detecting antibodies against plasma membrane antigens of a human neuroblastoma cell line (Sk-N-Mcl), and an immunocytochemical technique for detecting antibody binding to neurons in sections of fixed rat midbrain. Three of the PD CSF samples produced detectable staining of rat midbrain cells; no antibodies against neuroblastoma antigens or cardiacin was found in these samples. IL-1b, IL-2R, and TNF were not detected in PD CSF samples; B-2-M was detected in all PD CSF samples but did not differ from B-2-M in normal CSF. Our data do not indicate that local immune system activation is present in PD (Supported by the Student Fellowship Program of the Michigan Parkinson Foundation and NIH grants NS-27892, PAL, and NS-28081, GK).

The retina and substantia nigra arise from a common embryological source. Degenerative changes in both tissues occur in Parkinsonism. Retinal function may be assessed through the use of electroretinogram obtained under conditions of light and dark adaptation. Some evidence suggests that sleep deprivation (SD) may improve the motor dysfunction of Parkinsonism. The present study sought to determine if similar changes also occur in retina. Motor function and ocular potentials were examined in patients with Parkinson’s disease prior to and following SD. This treatment substantially improved motor function (>40%) and significantly increased ocular potentials. Improvements in these measures were highly correlated (r=0.9) and were approximately equally distributed across the range of the group response. The results confirm that SD therapy may provide temporary relief of motor disabilities. Parallel actions in retina and striatum, and the anatomical isolation of these tissues, further suggest that the SD effect may be mediated through induction of an endogenous humoral agent. Such an agent could provide a basis for the development of more effective treatments for Parkinson disease.

PATIENTS WITH HEPATIC ENCEPHALOPATHY SHOW ALTERATIONS OF BRAIN ACTIVITY THAT APPEARS TO RELATE TO DOPAMINERGIC SYSTEMS. J. R. McConnell*, H.A. Scott*, A. D. White, J. R. McConell*, J. R. McConell*, J. R. McConell*, and C. R. Green*. Departments of Anatomy, Radiology and Psychiatry, University of Nebraska Medical Center, Omaha, NE 68198.

Hepatic Encephalopathy (HE) is an alteration in mental state due to chronic liver failure. Although the etiology of HE is varied, the expression of the disease is fairly typical from subtle changes in the intellect to profound intellectual and motor function to coma and death. During the progression of the disease we have noted that the patient displays signs of agitation with accompanying extrapyramidal signs of activity. We have investigated whether HE patients might have some changes in their extrapyramidal system by using Magnetic Resonance Imaging (MRI) in 4 patients diagnosed with HE. The HE brain limbs were compared to the MRIs of patients that did not have HE. All the images were scanned and the reflectances were analyzed by density and with the use of Neuromagnetic Software system. Our results show that there were significant differences in areas of the extrapyramidal system, but that in addition some major fiber tracts were also affected: particularly the corpus callosum and the lateral geniculate area showed a significant effect using both axial (A) or sagittal (S) approaches. There were no significant differences in areas of the central nuclei, nor in the thalamus. There were some changes in the hippocampus and anterior internal capsule, but due to our small number of patients, the differences were not statistically significant. It appears that the HE patient is experiencing some alteration in the extrapyramidal pathways as well as in a mesothalamo-pyramidal pathway. It remains to be determined whether the small effects in the hippocampus can be replicated as they would explain some of the behavioral deficits. This work was partially supported by a grant from the HO-12319 and funds from the University of Nebraska Medical Center Department of Radiology.

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499.11


Groups of patients with idiopathic Parkinson’s disease (PD), either medicated or unmedicated, were compared with matched controls on a computerised battery of tests designed to assess different aspects of visuo-spatial memory and learning. In a test of spatial working memory known to be sensitive to frontal lobe damage, significant impairments were found only in the medicated PD patients and particularly in those patients with more severe clinical symptoms. These medicated patients ‘later in the course’ of the disease were also impaired in both a delayed matching to sample procedure and at learning the location of a number of patterns on the screen. In contrast, spatial recognition was significantly impaired in medicated PD patients with both mild and severe clinical symptoms.

In a parallel experiment, the same battery of tests was given to 10 PD patients ‘on’ and ‘off’ L-Dopa therapy. Significant impairments following withdrawal of medication were only evident in the test of spatial working memory.

These results suggest that there are multiple memory impairments in PD which may differentially depend on the clinical severity of the disease. In addition, the selective impairment in spatial working memory following L-Dopa withdrawal suggests that those tests of memory function most sensitive to frontal lobe damage also depend most heavily on dopaminergic substrates.

499.13


Pergolide mesylate, [(Methylthio)methyl]-6-propargyl monomethane-sulfonate (=Y127809), is a synthetic ergot alkaloid with potent dopaminergic (D1 and D2) activity and is marketed under the trade name Permax® for use in the treatment of Parkinson’s Disease. The low level of pergolide found in plasma (<1 ng/ml) after oral administration complicated the development of an analytical methodology for pergolide. Accordingly, we developed and validated a monoclonal antibody-based competitive RIA specifically optimized for the measurement of pergolide in plasma (Table I). Briefly, samples (0.5 ml) are incubated 20 h at 10 C with 75 ng of tritiated pergolide (0.1 ml) and anti-pergolide MoAb diluted 1:50,000 (0.1 ml). Separation of bound and free labelled pergolide is achieved by adsorbing the free fraction with 1% dextran-coated 5% charcoal. Plasma levels are then estimated from a standard curve of reference pergolide ranging from 5 to 5,000 pg/ml.

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<thead>
<tr>
<th>Sample volume</th>
<th>Sensitivity</th>
<th>Recovery</th>
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<tr>
<td>25 pg/ml</td>
<td>168 pg/ml</td>
<td>82-97%</td>
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Displacement experiments involving 4 pergolide metabolites found during animal studies and 5 synthetic analogs indicated the RIA is specific for pergolide and permitted defining the MoAb binding epitope. We conclude the RIA is a valid method for quantifying therapeutic concentrations of pergolide in plasma from man, rat and monkey.
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500.1

The mechanism or effects of acceleration induced loss of consciousness (G-LUC) observed in pilots of high performance aircraft are not fully understood. It has been proposed that cerebral blood flow may cease due to zero carotid artery pressure within 2s after >5 +Gz exposure. HYPOTHESIS: GM-LUC may result from global ischemia and subsequent energy depletion. METHODS: A SAE equipped with a 2450 MHz, 10 KW, MW system with rotating waveguide was used for brain fixation. Mice were exposed to either varying +Gz (15 to 35) forces or duration (U-35s) and brains were fixed at specific time points during and 60s post run. brain glycolytic and high energy phosphates were measured. RESULTS: The 6-tolerance data show that the levels of glucose, C-P and ATP decreased and lactate accumulated as +Gz force increased. The time curve shows significant changes in glucose (-85%), C-P (-73%) and ATP (-20%), lactate (+220%), AMP (+76%) and adenosine triphosphate (+12 fold) 35s after the onset of 35 +Gz. The level of these metabolites were not restored 60s after the SAC run.
CONCLUSIONS: (1) MW is an ideal tool for brain fixation during +Gz exposure. (2) G-LUC may occur to conserve energy resources during the cessation of cerebral blood flow in a high +Gz environment.

500.2

EFFECT OF LOW FREQUENCY PULSING ELECTROMAGNETIC FIELDS (PEMFs) ON FOCAL CEREBRAL ISCHEMIA. G.A. Grant, R. Cadoni and G.R. Steinberg. Dept. of Neurosurgery, Stanford Univ. Sch. of Med., Stanford, CA 94305.
There is evidence that electromagnetic stimulation may accelerate the healing of tissue damage following ischemia. We studied the effect of exposure to PEMFs on cerebral injury in a rabbit model of transient focal ischemia (2 hr. occlusion of left ACA, ICA, and MCA, followed by 4 hr. of reperfusion). The 32 animals were exposed (240 V, 75 Hz) was initiated 10 min. after the onset of ischemia and continued through the reperfusion period. 6 control, 6 experimental. Magnetic resonance imaging (MRI) and somatosensory evoked potentials(SEPs) were used to measure the degree of ischemic injury. Exposure to the PEMF did not significantly reduce the area of MRI injury (exposed, 16.2 vs. control, 17.3). Preliminary data suggest that exposure to a PEMF of short duration has no beneficial role in the treatment of stroke, although another prolonged exposure may prove efficacious. (NIH Grant 881069 and IGEA).

500.3

PROTEIN OXIDATION AND INHIBITION OF GLUTAMINE SYNTHETASE IN THE CEREBRAL CORTEX FOLLOWING CARDIAC ARREST AND RESUSCITATION. G. Fiskum1, R.E. Rosenthal2*, and P.E. Starke-Reed3*, Dept. of Biochemistry and Molecular Biology1, Emergency Medicine2, and Psychiatry, Univ. of Minnesota Medical School, Minneapolis, MN 55455 and 3 Dept. of Neurology, Psychiatry, Univ. of Minnesota Medical School, Minneapolis, MN 55455.
Decapitation-ischemia is known to cause a rapid hydrolysis of poly-phosphoinositides with concurrent increases in inositol phosphates, free fatty acids (FFA) and diacylglycerols (DAG) in brain. In this study, the effects of lithium administration on these events were examined in adult C57BL/6J mice with 240 V, 75 Hz PEMF exposure i.p. either lithium (8 meq/kg body wt) or saline 4 hr prior to decapitation and then subjected to decapitation ischemic treatment (5, 35, 65, 125, 305 sec). The cerebral cortex was taken for analysis of inositol phosphates by ion chromatography, inositol(1,4,5)P3, followed by rapid conversion to inositol(1,4)P2 and inositol(4)P with peak activities at 35, 65 and 125 sec, respectively. Lithium administration did not alter the turnover of inositol(4)P but increased the levels of inositol(1,4,5)P3 and inositol(4)P. Decapitation resulted in an immediate increase in the levels of DAG but the increase in FFA levels showed a lag time of 35 sec. Lithium treatment resulted in a decrease in the initial phase of FFA release which was reflected in an increase in the level of DAG. These results suggest that lithium may interfere with the release of FFA in brain through the DAG-lipase pathway.

500.4

Decapitation-ischemia is known to cause a rapid hydrolysis of poly-phosphoinositides with concurrent increases in inositol phosphates, free fatty acids (FFA) and diacylglycerols (DAG) in brain. In this study, the effects of lithium administration on these events were examined in adult C57BL/6J mice with 240 V, 75 Hz PEMF exposure i.p. either lithium (8 meq/kg body wt) or saline 4 hr prior to decapitation and then subjected to decapitation ischemic treatment (5, 35, 65, 125, 305 sec). The cerebral cortex was taken for analysis of inositol phosphates by ion chromatography, inositol(1,4,5)P3, followed by rapid conversion to inositol(1,4)P2 and inositol(4)P with peak activities at 35, 65 and 125 sec, respectively. Lithium administration did not alter the turnover of inositol(4)P but increased the levels of inositol(1,4,5)P3 and inositol(4)P. Decapitation resulted in an immediate increase in the levels of DAG but the increase in FFA levels showed a lag time of 35 sec. Lithium treatment resulted in a decrease in the initial phase of FFA release which was reflected in an increase in the level of DAG. These results suggest that lithium may interfere with the release of FFA in brain through the DAG-lipase pathway.

500.5

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500.6

PERSISTENCE OF SEROTONIN AND CHOLECYSTOKININ IMMUNOREACTIVE NERVE FIBERS IN THE GERBIL HIPPOCAMPAL FORMATION FOLLOWING CEREBRAL ISCHEMIA T. Kodok, J. Howard, P. Paris, P.K. Hartmann, Y.J. Li1, S.M. Onifer3, and W.C. Low1. Depts. of Neurosurgery1, Psychiatry, Univ. of Minnesota Medical School, Minneapolis, MN 55455 and 3 Dept. of Neurological Surgery, Univ. of Miami School of Medicine, Miami, FL 33101.
Serotonin (5HT) and cholecystokinin (CCK) -immunoreactive nerve fibers were studied in the hippocampal formation following the induction of transient forebrain ischemia in the mongolian gerbil. Transient cerebral ischemia was produced by occluding bilaterally the carotid arteries for a period of 10 min. Nerve fibers within the ischemic hippocampus were visualized using antibodies raised against 5HT and CCK. Pyramidal neurons in the dorsal CA1 region of the hippocampal formation had degenerated extensively 6-12 months after the ischemic episode. 5HT- and CCK-immunoreactive fibers were found to persist throughout the ischemic CA1 field in the absence of their post-synaptic targets. These observations suggest that 5HT and CCK fibers are resistant to ischemic injury. Moreover, the persistence of scar tissue suggests that they may be available to establish host-to-graft fiber connections with transplanted hippocampal pyramidal cells in attempts to reconstitute neural circuits that are damaged following ischemic injury.
HYDROCEPHALUS. U.S. Vasthare*, J.P. McAllister, P.M. Hale*. Anatomy and Neurosurgery, Temple University School of Medicine, Philadelphia, PA 19140.

This study was designed to assess the effects of infantile hydrocephalus and surgical decompression on cerebral blood flow (CBF) and somatosensory evoked response (SSER). Hydrocephalic rats were induced on day 10 old by injection of cisternal injection of kaolin and verified with ultrasound; saline-injected or normal animals served as age-matched controls. Radiolabeled microspheres were used to measure CBF in severely hydrocephalic animals; standard SSER measurements were performed on control and hydrocephalic animals, as well as hydrocephalic animals that were decompressed with ventriculoperitoneal (VP) shunts at 6–13 days post–kaolin. Significant (p less than 0.05) decreases in CBF were detected in cortical areas (61% below control), 22 (54%) and 17 (55%), as well as the thalamus (46%), midbrain/pons (54%) and caudate nucleus (64%); non–significant decreases occurred in the hippocampus (41%) and cerebellum (40%). Since mean CBF never fell below 23 ml/min/100g in any area, ischemia may not play a major role during hydrocephalus. Hydrocephalic exhibited pre–shunt increases in the SSER cortical peak latency, which was not reversed immediately by VP shunting. Latency did return to normal by 7 days post–shunt. This early recovery suggests that axons are not structurally impaired during hydrocephalus.

BLOOD FLOW THRESHOLD FOR ISCHEMIC DEPOLARIZATION OF RAT NEOCORTEX Y. Takeda*, M. Jacewicz and W. Pulsinelli. Dept of Neurology and Neuroscience, Cornell University Medical College, New York, NY.

To determine the ischemic blood flow threshold below which rat neocortex depolarizes, we implanted a DC potential electrode (DCPE) in the neocortex of halothane–anesthetized Spontaneously Hypertensive rats (n=27) and occluded their middle cerebral (MCA) and posterior common carotid (CCA) arteries. The DCPE was inserted 800 μm below the cortical surface at 2 mm posterior and 3 mm lateral to bregma. This location represents a previously-determined transition zone between moderate and mild ischemia. To assure the second–to–second stability of CBF in the vicinity of the DCPE, a laser–Doppler flow probe was positioned within 1 mm of the DCPE. Approximately 10 minutes after MCA/CCA occlusion 14C–iodoantipyrine (14C–IAP) was injected intravenously and the animals were depleted 5 minutes later. CBF was measured at the site of the DCPE using autoradiographs of frozen, cross–coronal brain sections and image analysis (Quantix, Dualegt) 907). The DCPE site was located by injecting 0.15 μl of fluorescein (0.9 mg/ml) through the DCPE just prior to injecting 14C–IAP. Statistical analysis of 27 rats (13 with depolarization, 14 without) using logistic regression and the jack–knife method indicated that 10% of rats depolarized at a CBF of 7.2 ± 5.3%, 50% at 47.8 ± 1.5% and 90% at 22.2 ± 3.0 ml/100 gm/min. The results are consistent with our observations that the CBF in the border zone of focal cerebral infarction is approximately 45–50 ml/100 gm/min (Jacewicz et al, Soc. Neurosci. Abst., 16:1277, 1990). We conclude that the threshold for ischemic depolarization in the SHF and therefore the threshold for ischemic brain injury in this species lies at approximately 50 ml/100 gm/min.

CEREBRAL ISCHEMIA IN GERBILS: THERAPEUTIC EFFECTS OF DELAYED TREATMENT WITH CALCIUM ANTIDOTE CALCIUM-PQ (DIMER OF 16/16 DIETHYL 4,5-DIETHYL-1,2-PROPANEDIOL) D.E. Cooper*, B. E. Szymanski, R. T. Cooper, P. V. Smelser. Department of Neurology, University of Virginia Health Sciences Center, Charlottesville, VA.

We demonstrated previously that immediate postischemic application of Di-Calciphor resulted in a pronounced improvement in survival as well as enhanced quality of neurological recovery. We now describe the actions of Di-Calciphor given I.P. at 5 or 10 mg/kg at 30, 60 or 180 min and 24 hrs postischemia (N=25/group). Ischemic controls (N=25) were injected with the vehicle. A group of 50 male Sprague-Dawley rats were subjected to 10 mins of bilateral carotid artery occlusion moderate 14C-iodoantipyrine (14C–IAP) was injected intravenously and the animals were depleted 5 minutes later. 14C–iodoantipyrine (14C–IAP) was injected intravenously and the animals were depleted 5 minutes later. The DCPE site was located by injecting 0.15 μl of fluorescein (0.9 mg/ml) through the DCPE just prior to injecting 14C–IAP. Statistical analysis of 27 rats (13 with depolarization, 14 without) using logistic regression and the jack–knife method indicated that 10% of rats depolarized at a CBF of 7.2 ± 5.3%, 50% at 47.8 ± 1.5% and 90% at 22.2 ± 3.0 ml/100 gm/min. The results are consistent with our observations that the CBF in the border zone of focal cerebral infarction is approximately 45–50 ml/100 gm/min (Jacewicz et al, Soc. Neurosci. Abst., 16:1277, 1990). We conclude that the threshold for ischemic depolarization in the SHF and therefore the threshold for ischemic brain injury in this species lies at approximately 50 ml/100 gm/min.

ACUTE HYPERGLYCEMIA ON POSTISCHEMIC MORTALITY IN THE RAT. T.A. Cicerone*, D. Warnacott, M.M. Todd. Department of Anesthesiology, University of Iowa, Iowa City, IA 52242.

Acute hyperglycemia (i.e., i.v. glucose infusion) prior to global ischemia often results in brain edema, seizures and death. To determine if chronic pre–ischemic hyperglycemia has similar effects on mortality, 3 groups of male Sprague-Dawley rats were subjected to 10 mins of bilateral carotid artery occlusion moderate 14C-iodoantipyrine (14C–IAP) was injected intravenously and the animals were depleted 5 minutes later. 14C–iodoantipyrine (14C–IAP) was injected intravenously and the animals were depleted 5 minutes later. The DCPE site was located by injecting 0.15 μl of fluorescein (0.9 mg/ml) through the DCPE just prior to injecting 14C–IAP. Statistical analysis of 27 rats (13 with depolarization, 14 without) using logistic regression and the jack–knife method indicated that 10% of rats depolarized at a CBF of 7.2 ± 5.3%, 50% at 47.8 ± 1.5% and 90% at 22.2 ± 3.0 ml/100 gm/min. The results are consistent with our observations that the CBF in the border zone of focal cerebral infarction is approximately 45–50 ml/100 gm/min (Jacewicz et al, Soc. Neurosci. Abst., 16:1277, 1990). We conclude that the threshold for ischemic depolarization in the SHF and therefore the threshold for ischemic brain injury in this species lies at approximately 50 ml/100 gm/min.

EFFECTS OF ACUTE VERSUS CHRONIC PRE-ISCHEMIC HYPERGLYCEMIA ON POSTISCHEMIC MORTALITY IN THE RAT. T.A. Cicerone*, D. Warnacott, M.M. Todd. Department of Anesthesiology, University of Iowa, Iowa City, IA 52242.

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500.13

The effects of bilateral common carotid artery occlusion on local cortical perfusion and forebrain histopathology were examined in CFW mice. Under chloral hydrate anesthesia transient bilateral occlusion of the common carotid arteries elicited an immediate and sustained reduction in local cortical perfusion (95%) which was reached within 1 min and was maintained throughout the occlusion period (5 to 10 min). Similarly, the concomitant deterioration of the EEG power was reached a minimum (92.7%) within 1 min and remained depressed during ischemia. A histopathologic evaluation of the forebrain performed 7 days following 5 and 10 min periods of bilateral common carotid artery occlusion revealed significant neuronal damage in the cortex, dentate, and CA1 and CA3 fields of the hippocampus. The degree of damage in these areas were related to the duration of the ischemia. A carbon black study of the posterior communicating arteries in 16 mice indicated that 25% had patent arteries bilaterally, 37.5% had only one patent artery and 37.5% had no patent arteries. We conclude that the posterior cerebral circulation in the CFW mouse provides inadequate forebrain perfusion following bilateral common carotid artery occlusion. Hence, the CFW mouse may provide a useful model for the study of transient forebrain ischemia.

500.15

Abnormal elevations in intracellular calcium has been strongly implicated in the neurodegeneration that follows brain ischemia. Moreover, disassembly of the neurons' cytoskeleton has recently been shown to be an early event in their neuronal death, while activation of calcium-mediated proteases (calpains) appears to represent a final common pathway for the neurons' destruction. For these reasons, inhibition of calpain provides a novel and potentially powerful means of reducing neuronal death due to other neurotoxic perturbations.

Several novel calpain inhibitors have been identified which are more potent, selective and efficacious than standard compounds. We evaluated the ability of these compounds to protect hippocampal neurons from transient global ischemic damage induced by ischemia alone and to its calcium-mediated protease activity. These studies demonstrated the superior efficacy of the novel, non-aldehyde inhibitors, providing more effective protection of pyramidal cells. They therefore provide the clearest evidence, to date, that despite the likely involvement of other events in the pathogenic cascade, inhibition of calpain is sufficient to protect a large number of vulnerable hippocampal neurons from the damaging effects of ischemia. Moreover, these data provide the first evidence that complete protection of certain hippocampal neuronal populations can be achieved, even when the inhibitors are administered well after the ischemic event is over.

500.16

Activation of cyclic nucleotide dependent protease calpain has been implicated in long-term potentiation, excitatory amino acid-induced neurodegeneration and ischemic brain damage. This study was conducted to evaluate the efficacy and pharmacological profile of four novel non-aldehyde calpain inhibitors (CX216, CX217, CX218 and CX233) versus the standard aldosterone inhibitors, Calpain Inhibitor 1 (C1) and Leupeptin (LEU) in protecting synaptic transmission in hippocampal slices from hypoxia.

Hippocampal slices (400 µm) were maintained in chambers perfused with artificial CSF and O2 5% CO2. Hypoxic exposure nitrogen for 30 min and reoxygenation for recovery of synaptic transmission from hypoxia were measured.

While the standard aldosterone calpain inhibitors were only modestly effective in protecting hippocampal slices from hypoxia, all four novel non-aldehyde inhibitors were found to be more potent, efficacious and membrane permeant. For example, 100 µM of all four CX compounds, preincubated for 30 minutes or 1 hour, produced a 40% to 80% survival rate, respectively, compared to 40% survival rate, respectively, of 2128 (100 µM) and 1 hour preincubation improved the recovery of synaptic transmission to 62% following hypoxia. In contrast, C1 and LEU were ineffective under similar conditions. C1 required 200 µM with a 2 hour preincubation period, while LEU required 1 mM with a 3 hour preincubation period to provide even modest protective effects. These results provide clear evidence that inhibition of calpain can reliably ameliorate functional damage in hippocampal slices induced by hypoxia. Moreover, recently developed non-aldehyde inhibitors are much more effective than the commercially available aldosterone inhibitors.

500.17

Adenosine has been characterized as a natural modulator of glutamate neurotransmission and adenosine analogs have therefore been explored as possible therapeutic agents for excitotoxic amino acid toxicity. However, attempts to develop effective peripheral administered adenosine agonists for neuroprotection have been confounded by two key issues. The first is that at efficacious doses, moderate side effects (i.e., hypothermia, sedation, hypotension) are seen. These side effects, alone or in combination with effects on synaptic transmission, can protect neurons from the damaging effects of global ischemia. The second issue is whether adenosine agonists even penetrate the blood-brain barrier (BBB). It has been suggested that the neuroprotection attributed to adenosine agonists is due primarily to their peripheral side effects rather than a direct effect on central neurons.

In a series of experiments, we addressed a number of issues related to these problems. We have shown that agents which are moderate adenosine agonists are administrated centrally (IV), hypothermia and sedation are still observed, but neuronal damage is significantly reduced. A portion of neuronal damage in several models of global ischemia. The second issue is whether adenosine agonists even penetrate the blood-brain barrier (BBB). It has been suggested that the neuroprotection attributed to adenosine agonists is due primarily to their peripheral side effects rather than a direct effect on central neurons.

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Prompt dendritic damage and its central propagation have been observed in the periphery of dendrites in the gerbil hippocampus after transient cerebral ischemia. We examined the frontoparietal cortex of gerbils after bilateral carotid occlusion for 20 min and reperfusion for 72 hrs. During ischemia without reperfusion, progressive swelling of the periphery of dendrites with swelling of mitochondria, disintegration of microtubules and formation of microvacuoles occurred in layer I and less notably in layers II and III. After reperfusion for 3 hrs, swelling of dendritic terminals and mitochondria receded but disintegration of microtubules and formation of microvacuoles propagated to deeper layers. Swelling of glial processes also became apparent in many layers after 3 hrs, causing indentation in dendrites. Neuronal perikarya showed disaggregation of polynucleosomes and occasional fragmentation of rough endoplasmic reticulum after reperfusion for 3 hrs. Disintegration of mitochondrial cristae and formation of microvacuoles occurred after 6 hrs, particularly in layers III and Vb, and some neurons became clumped and electron-dense. While the number of these degenerated neurons increased progressively, surviving neurons with reaggregation of polynucleosomes also increased beyond reperfusion for 12 hrs. The present study showed central propagation of dendritic damage and progressive structural disintegration of neuronal perikarya before "delayed neuronal death" became apparent with light microscopy.

**ISCHEMIA: EXCITOTOXICITY**

**501.1**

**NECESSITY OF CONTINUED INFUSION FOR NEUROPROTECTIVE EFFECT OF NMDA ANTAGONISTS IN CEREBRAL ISCHEMIA**

D.M. Kunis, G.H. Sun*, A. Poljak* and G.K. Steinberg

Dept. of Neurosurgery, Stanford University, School of Medicine, Stanford, CA 94305

While both competitive and noncompetitive NMDA antagonists have been shown to protect against focal cerebral ischemia, it is not clear whether a single dose or continued maintenance infusion of these drugs is necessary to achieve neuroprotection. We studied this issue using dextromethorphan (DM), dextroamphetamine (DX), and MK801 in a rabbit model of transient focal ischemia. Thirty rabbits underwent two hour occlusion of the left internal carotid, anterior cerebral, and middle cerebral arteries followed by four hours of reperfusion. Ten minutes after occlusion, the rabbits were treated with DM 20 mg/kg or 20 mg/kg followed by 10 mg/kg/dx, DX 16.7 mg/kg or 16.7 mg/kg followed by 8.35 mg/kg/dx, MK801 1 mg/kg or 1 mg/kg followed by 0.75 mg/kg/dx or normal saline. The loading dose was given over 30 minutes. Ischemic injury was assessed using somatosensory evoked potentials, magnetic resonance imaging and histopathology. For DM and DX, maintenance infusions were necessary to achieve neuroprotection, whereas a loading dose only was protective for MK801. Maintenance infusion of MK801 tended to worsen ischemic injury, possibly by lowering blood pressure. The need for continued dosing of NMDA antagonists may depend on their serum and brain half lives.

Supported by AHA grant 881069, NIH grant R01 NS 27292-01A2, and the Valerie K. Bernhard Cerebral Ischemia Fund.

**501.4**

**EXTRACELLULAR pH AND K+ GRADIENTS IN FOCAL ISCHEMIA OF RAT CORTEX**

M. Chelier and Z. Hassan

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Decreases in extracellular pH (pHc) below 6.6 abolish NMDA-gated currents (1). Thus, in ischemia, the behavior of pHc may determine the extent of excitotoxic injury. We studied pHc in rat cortex following middle cerebral artery (MCA) occlusion. Non-fasted, female, Long Evans-Rooded rats were anesthetized with pentobarbital, paralyzed and ventilated on O2-supplemented air. pHc microelectrodes were inserted through a 300 μm hole in a silicone sheet, covering a 2 mm parietal craniotomy. Control pHc was 7.31 ± 0.02 with no cortical gradient. Mean arterial pH, pCO2 and O2 (torr) were 7.41/59/130 (pre-ocl.) and 7.40/38/82 (post-ocl.) respectively. Within 15 min of MCA occl., minimum pHc decreased by 0.20 ± 0.13 pH units/mm depth. Below the point of maximum acidity, pHc decreased by 0.57 ± 0.13 pH units/mm depth, with mean pHc of 6.94 ± 0.19 as 2000 mm. Decreases and increases in pHc were accompanied by neg. and pos. shifts in DC potential respectively. Preliminary measurements of (K⁺) revealed similar U-shaped gradients, with troughs at upper and lower depths. These data indicate that in non-fasted rats, MCA ocll. can cause a pHc fall sufficient to protect against NMDA receptor-mediated injury. Vulnerability of superficial neurons may be related to higher pHc. Proximity of CSF, preservation of blood flow and CO2 egress might account for these gradients, however, their cause remains to be determined. (1) Tang, C.M. et al. (1990). PNAS, 87(16):6445. Supported by NINDS Grants NS10164 and NS23701.

NMDA antagonists reduce neuronal vulnerability to hypoxic neuronal injury both in vivo and in vitro. However, prior to in vitro studies have taken place at normal pH, whereas hypoxia–ischemia in vivo is associated with extracellular acidity, a perturbation that attenuates NMDA receptor activation, glutamate neurotoxicity, and hypoxic injury in vitro. We investigated whether acid pH would abolish the value of glutamate antagonists against a combined oxygen– and glucose-deprivation insult in murine cortical cell cultures.

Near-complete protection from insults lasting up to 60 min was produced by dropping extracellular pH to 6.4. The neuronal death produced by longer insults (80–120 min) was still reduced by the addition of the NMDA antagonists MK-801 or CGS-19755. Even further injury reduction was produced by addition of CNQX to saturating concentrations of MK-801, consistent with earlier reports of synergy at normal pH. Thus whereas brief exposure to extracellular acidity was neuroprotective, additional protective effects were produced by glutamate antagonists. When both NMDA and AMPA/kainate receptors were maximally blocked, dropping pH to 6.4 produced additional injury reduction. Extracellular acidity may have neuroprotective effects in hypoxic neuronal injury independent of glutamate neurotoxicity.

501.7 COMBINED TREATMENT WITH MK-801 AND A D1/D2 Dopamine Antagonist Reduces Cerebral Ischemic Damage. S. M. Nurse*, D. Corbett and S.J. Evans. Basic Medical Sciences, Fac. of Medicine, Memorial Univ., St. John's, NF, Canada, A1B 3V6.

During ischemia extracellular levels of both dopamine (DA) and glutamate rise substantially, an event which may contribute to ischemic injury. This study examined the therapeutic efficacy of DA antagonists alone and in combination with MK-801.

Global ischemia was induced in gerbils by 5 min carotid artery occlusion at 38°C. MK-801, 5 mg/kg, was given 1 hr prior to surgery; cis-flupenthixol, 0.4 and 1.2 mg/kg, 3 hr prior to surgery; and SCH-23390, 1 mg/kg, 0.5 hr prior to surgery. MK-801 but not cis-flupenthixol reduced CA1 cell loss. Together these drugs were slightly more effective than MK-801 alone. SCH-23390 was ineffective.

Combined drug therapy with D2 DA and NMDA antagonists may be a useful treatment strategy in cerebral ischemia. Supported by the MRC of Canada.

501.9 ISCHAEMIA INDUCED RELEASE OF GLUTamate, ASPARtate AND GABA FROM RAT RETINA IN VITRO POSSIBLE IN VIVO. V. Van Vlem, J.M. Neel and J.K. Cunningham, Dept. Pharmacology, St. Thomas's Hospital, London, SE 1 EH, UK.

We have examined the effects of 'ischaemia' on the release of amino acids from the rat retina in vitro. Isolated retinas (dark-adapted or exposed to room light) were incubated at 32°C in Krebs bicarbonate medium gassed with 95% O2/5% CO2 (controls) or in glucose-free medium gassed with 95%N2/5% CO2 (ischaemia). Retinas were incubated for 10–90 min and the amino acids in the medium measured by HPLC.

When control retinas were incubated in the light for periods up to 90 min there was a release of (GLU) from the retina. Asp and GABA (1.5±0.2, 0.10±0.026; 2.15±0.15 mmol/retina/30 min respectively, mean ±SEM n=5). Release was similar from control dark-adapted retinas. When control retinas were incubated in medium containing 30 mg/ml of the glutamate antagonist, MK-801, there was a significant reduction in both the amount of GLU and ASP released. The release of GLU, ASP and GABA from retinas incubated in the light under ischemic conditions for 90 min was increased 14.0, 19.1 and 9.3 fold respectively. The release of GLU and especially ASP from dark-adapted retinas incubated under ischemic conditions was significantly higher than that from retinas in the light.

Remarkably, the ischemia–induced release of GLU, ASP and GABA from retinas incubated in the light was not Ca-dependent, whilst the release from dark-adapted retinas was reduced by approximately 60% in high-Mg2+/low-Ca medium. The NMDA antagonist in murine cortical cell cultures. Near-complete protection from insults lasting up to 60 min was produced by dropping extracellular pH to 6.4. The neuronal death produced by longer insults (80–120 min) was still reduced by the addition of the NMDA antagonists MK-801 or CGS-19755. Even further injury reduction was produced by addition of CNQX to saturating concentrations of MK-801, consistent with earlier reports of synergy at normal pH. Thus whereas brief exposure to extracellular acidity was neuroprotective, additional protective effects were produced by glutamate antagonists. When both NMDA and AMPA/kainate receptors were maximally blocked, dropping pH to 6.4 produced additional injury reduction. Extracellular acidity may have neuroprotective effects in hypoxic neuronal injury independent of glutamate neurotoxicity.

501.10 THE EXCITOTOXICITY OF QUINOLINIC ACID IN HYPOLACRINE RATS. A. Scharf, A. C. Wood and S. M. Black. Dept. of Anesthesiology, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

Quinolinic acid (2-pyridyl diketobutyric acid, QA), an NMDA receptor agonist, has been shown to induce excitatory, neurotoxic and convulsant effects in both mice and rats. It is the most potent endogenous excitotoxin known to date.

The aims of this study were to explore the structural essentials of QA agonistic properties and to define certain conditions that potentiate both excitotoxicity and convulsant effects. Orthodromically evoked CA1 population spikes were recorded from rat hippocampal slices. QA's ability to enhance excitatory neuronal damage in vivo under conditions of ischemia (response after 30 min reoxygenation) was compared with that of different QA derivatives. In addition, the effect of changes in the concentration of several ions in the perfusion medium on the excitotoxicity of QA was tested.

QA enhanced excitatory neuronal damage in dose-dependent fashion and an effect that was blocked by the NMDA antagonist APV. None of the other pyridine diketobutyric acids tested could enhance hypoxic damage. Doublet of NMDA and QA were not additive. Neither Ca2+ nor Mg2+ play a role in QA excitotoxicity.
501.11
CYS TEA CID AC E D E N H A N C E S H Y P OX IC AND H Y P O G LY CE M IC N E U R A L DAMAGE IN VITRO. C.A. West*, A. Schurr and B.M. Rigor, Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292.
L-Cysteic acid (CYS) is classified as a relatively weak analog of MMA in terms of its excitotoxic potency. We have tested the rat hippocampal slice preparation and its electrophysiology as the in vitro system most suitable for studies on the involvement of excitotoxins in the mechanism of hypoxia- and hypoglycemic neuronal damage. The combined effects of CYS and compromised energy metabolism on the degree of neuronal damage is the focus of this presentation.

Under normoxic and normoglycemic conditions (95% O2:5% CO2:1% O2) neuronal damage, severe hypoglycemia was surprisingly mild at 1 mM. However, this concentration of CYS was harmful at 3 mM glucose. 30 min reoxygenation or 15 min hypoglycemia reduced the recovery rate of neuronal function (evoked, CA1 orthodromic population spike) after either 10 min hypoxia (95% N2) and 30 min reoxygenation or 75 min hypoglycemia (0 mM glucose) and 30 min glucose repletion in a dose-dependent fashion. At 0.2 mM the NMDA antagonist APV did not block the effect of CYS on hypoxic slices.

The present results indicate that CYS is as potent as glutamic and aspartic acid in enhancing either hypoxic or hypoglycemic neuronal injury and re-oxygenation induced release of glutamate were decreased significantly by adding 20 μM CYS before or after hypoxia. The evoked (electrical stimulation, 4Hz, 2 min) release of H+ glutamate was halved by CYS; this decrease was reversed by 20 μM phenytoin. These results indicate that phenytoin reduces hypoxia-induced release of the excitatory amino acid glutamate, while allowing cholinergic neurons to remain functional. Thus, phenytoin may protect neurons from stroke-induced toxicity and may also reduce re-perfusion injury and delayed neuronal damage. Supported by the Health Foundation.

501.12
PROTECTIVE EFFECT OF CCK-8 AND CERULETIDE AGAINST GLUTAMATE AND ISCHEMIA-INDUCED NEURONAL DAMAGE. G. Kasanuma, Y. Shinohara, M. Iiyoshi*, H. Shinok*, T. Shimot* and A. Matsushita*. Shionogi Res. Labs., Osaka 553, Japan
Glutamate is now recognized to play a pivotal role in learning/memory processes and neuronal damage. Recently, there is increasing evidence suggesting the involvement of glutamate and neuropeptides. In the present study we examined the effect of CCK-8 and ceruletide (CLT) on glutamate-induced neuronal death in neuron cultures and neuronal damage in the hippocampus of rat during ischemia. By means of LDH efflux, CCK-B (>10-10 M) and CLT (>10-13 M) markedly prevented glutamate (5x10-10 M)-induced neuronal cell death in neuron cultures. Gastrin-T (>10-8 M) showed the same effect. The response of CLT was completely blocked by an antagonist for CCK-B receptors, (1+1-365, 260). These peptides significantly suppressed the increase in cytosolic free Ca2+ levels induced by glutamate. CCK-B (10-10 M) and CCK-B (10-9 M) and CCK-B (10-8 M) respectively.

501.13
BASIC FIBROBLAST GROWTH FACTOR (BFGF) PROTECTS AGAINST ISCHEMIC NEURONAL DEATH IN VIVO. D.J. Berlove, C.G. Caldas*, R.A. Moschkowitz and S.P. Finklestein, Massachusetts General Hospital, Boston, MA 02114.
Basic fibroblast growth factor (BFGF) promotes neuronal survival and blocks excitatory amino acid (EAA) toxicity in CNS neurons in vitro. EAA toxicity is thought to play a major role in delayed neuronal death after cerebral ischemia in vivo. We tested the neuroprotective effects of BFGF after delayed (3 min.) global forebrain ischemia in the rat. Core temperature was kept constant during and after surgery. Animals were untreated, or received vehicle (artificial CSF) alone, or vehicle plus BFGF (1.2 μg/day) through continuous infusion via osmotic pump into the right lateral ventricle. Animals were observed for 3 days before and 7 days after ischemia. The degree of neuronal damage was scored on a 0-3 scale (0=no damage, 3=severe damage).

Recovery from hypoxia in vivo was assessed following temporal exposure to hypoxia under normoxic and normoglycemic conditions (95% O2:5% CO2:1% O2) exposures were performed on mixed glial cultures in vitro were examined following temporal exposure to hypoxia under normoxic and normoglycemic conditions (95% O2:5% CO2:1% O2) exposures were performed on mixed glial cultures.

GLUTAMATE AND ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPAL SLICES. M. Moskal3 Depts. Anesthesiology and Neurosurgery, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY 10467, and Chicago Inst. Neurosciences and Neuroresearch, Chicago, IL.
The effects of the anticonvulsant drug diphenylhydantoin (phenytoin) on hypoxia-induced changes in release of H+glutamate and H+acetylcholine were studied in superfused rat hippocampal slices. Hypoxia (25 min perfusion with Krebs buffer gassed with 95% O2:5% CO2) caused a large release of glutamate which lasted for 15-20 minutes. Re-oxygenation caused a second large release of glutamate, which persisted for at least 20 minutes. Both the hypoxia and re-oxygenation induced release of glutamate were decreased significantly by adding 20 μM phenytoin before or after hypoxia. The evoked (electrical stimulation, 4Hz, 2 min) release of H+ acetylcholine was halved by hypoxia; this decrease was reversed by 20 μM phenytoin. These results indicate that phenytoin reduces hypoxia-induced release of the excitatory amino acid glutamate, while allowing cholinergic neurons to remain functional. Thus, phenytoin may protect neurons from stroke-induced toxicity and may also reduce re-perfusion injury and delayed neuronal damage. Supported by the Health Foundation.

501.14
PHENYTOIN ATTENUATES HYPOXIA-INDUCED CHANGES IN GLUTAMATE AND ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPAL SLICES. P.E. Potter1, P. Devrier2* and J.R. Moskal3* Depts. Anesthesiology and Neurosurgery, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY 10467, and Chicago Inst. Neurosciences and Neuroresearch, Chicago, IL.
Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2. Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical excitotoxicity. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with 95% O2:5% CO2:1% O2.
501.17
THE EFFECT OF NMDA ANTAGONISTS ON EARLY ULTRASTRUCTURAL CHANGES IN NEURONAL CULTURE AFTER HYPOXIA. M.I. Haber, E.W. Marcoux, A.W. Probert and M.A. Dominick. Departments of Pharmacology and Pathology and Experimental Toxicology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI, 48105.
Mixed cortical neuronal-glial cultures isolated from fetal rat brain were exposed to hypoxia for 5 hours. Some plates were treated with the competitive NMDA antagonist 4-(3-phosphonopropyl)-2-piperazinonecarboxylic acid (CPP) prior to hypoxic exposure. Cultures were fixed with 1% glutaraldehyde immediately after hypoxia and then processed for transmission electron microscopy. Early ultrastructural changes were evident in neurons immediately after hypoxia at a time when only slight neuronal swelling was seen at the light microscopic level. Glial cells showed no apparent damage at 5 hours. Swollen mitochondria with disrupted cristae and vacuolization of the neuronal cytoplasm were seen at 5 hours. Control cultures subjected to 5 hours normoxia showed none of these changes. These changes paralleled early changes reported in vivo shortly after ischemia. Neurons treated with CPP showed no swelling grossly and ultrastructurally were identical to normoxic neurons. These results demonstrate NMDA antagonists ability to prevent the early intracellular changes in neurons consequent to hypoxia.

501.19
Several lines of evidence implicate activation of NMDA receptors as a mechanism of ischemic neuronal necrosis and of delayed neuronal death. While it has been shown that post-ischemic activation of NMDA receptors confers neuroprotection, in vivo microdialysis experiments indicate that extracellular levels of glutamate return to normal after the end of ischemia. In this study, 15 min after a hypoxic-ischemic insult (bilateral carotid ligation and exposure to 6% O2 for 1 hr) in the 7-day-old rat, pups were injected i.c.v. with [3H]-MK-801, with or without a prior injection of cold MK-801, and were sacrificed 10, 30, or 60 min later. The brains were rapidly removed and the cortices were frozen. Binding was determined by the filtration technique. Three additional groups were examined: untreated control and pups decapitated 10 min after [3H]-MK-801 injection with the heads kept at either 37 or 0°C for up to 60 min before dissection. Both total brain concentrations and non-specific binding were linearly related to dose. In control pups specific [3H]-MK-801 binding was 0.551 ± 0.044 pmol/g wet weight after 10 min and rose to 0.905 ± 0.042 pmol/g after 60 min. Decapilation ischemia at 37°C induced a rapid increase in binding to 1.860 ± 0.092 pmol/g by 30 min (p<0.001 vs controls) while binding during decapitation at 0°C was similar to that of controls. Binding in the post-hypoxic/ischemic brains was 1.737 ± 0.060 pmol/g after 30 min (p<0.001 vs controls). These results indicate that ischemia induces a dramatic activation of NMDA receptors and that this activation persists in the post-ischemic state. This may account for the efficacy of post-ischemic neuroprotection by NMDA antagonists and for delayed neuronal necrosis.

501.20
EFFECT OF QUISQUALATE AND HYPOXIA ON NEURONAL CELL INJURY. J.A. Kelleher and P.H. Chan. University of California CNS Injury and Edema Research Center, Dept of Neurology, M-734, Box 0114 San Francisco, California 94143 U.S.A.
Excitatory amino acid receptors are coupled to both ionic channels and signal transducing mechanisms. Excitatory synaptic transmission is associated with an endogenous release of glutamate and aspartate at potentially neurotoxic levels which does not appear to be altered by 2-CA.

501.21
SYNAPTIC STIMULATION EXCERBATES HYPOXIC INJURY IN PENUMBRAL NEURONS. P.R. Trueblood, R.A. Wallis, K.L. Parkison and C.G. Wasterlan. Sepulveda VAMC, Deps. of Neurology and Neurosurgery, UCLA Sch. of Medicine, and Sepulveda, CA 91355.
Early physical therapy is recommended in cerebral ischemia, yet the effect of the resulting synaptic firing on survival of neurons in the ischemic penumbra has never been studied. We examined the effects of synaptic stimulation on hypoxic CA1 neurons unable to generate an evoked potential upon Schaffer collateral stimulation. After hypoxic disappearance of evoked responses, paired slices were stimulated at 2 or 20 Hz (for an average duration of 2.2 min, ± 0.3 SE) until no evoked potential was observed. The synaptic injury potential (HIP) in the 2 Hz slice. With the disappearance of the HIP in the 2 Hz slice, oxygenation was restored. 20 Hz slices showed significantly reduced recovery of oxygen consumption and of the AMPA receptor antagonist quisqualate (PS) amplitude (B ± 3.3) compared to 2 Hz slices which received less stimulation during hypoxia (64% ± 14.8). In a similar paradigm, slices receiving orthodromic stimulation showed a significant decrease in the mean percent CA1 PS amplitude recovery (11% ± 3.4) compared to slices receiving antidromic stimulation (83% ± 17.0), presumably because the latter releases neurotransmitter. In low calcium medium, increased stimulation did not increase hypoxic injury significantly. These results suggest that synaptic stimulation can be detrimental to hypoxic penumbra neurons and that this damage is mediated by transmitter release. Supported by the research service of the VA, the American Epilepsy Society and grant NS15351 from NINDS.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
502.1 ROLE OF INTRACELLULAR CALCIUM IN HYPOXIC/ISCHEMIC INJURY IN CULTURED ASTROCYTES. R. S. Goldman, Department of Neurology, College of Medicine, SUNY, Upstate Medical Center, Syracuse, NY 13210.

A substantial body of evidence suggests that an elevated level of intracellular calcium is associated with the neuronal response to hypoxia. I have investigated the role of intracellular calcium changes in the astrocytic response to hypoxia. Primary cultures of rat astrocytes were exposed to sodium cyanide (CN; 1mM), an inhibitor of oxidative metabolism, and to iothalamate (IAA; 0.25mM), an inhibitor of glutamine synthetase. For various lengths of time, the cultures were then washed, and incubated. Cell viability was determined by a two color fluorescence assay. Cell survival was related to duration of exposure to CN and IAA; all cells were killed by a 16 minute exposure. Survival was increased by similar exposure in the absence of extracellular calcium. Additionally, astrocytes were preincubated with the intracellular calcium chelator BAPTA-AM (5mM). This should cause hypoinsulting of any hypoxia-induced intracellular calcium increase. This also resulted in increased cell survival.

Relative changes in the intracellular calcium concentration of cultured astrocytes were assessed using digital fluorescence microscopy, and the calcium indicator dye, fluo-3. A 10 minute incubation with CN and IAA produced a rapid rise in intracellular calcium. Calcium levels did not return to baseline following washout. The rise in calcium was significantly inhibited by incubation in low calcium buffer. Cells preincubated with BAPTA-AM showed little change in intracellular calcium levels during the first 5 minutes. However, with continued exposure, calcium levels did increase.

These results indicate that elevated levels of intracellular calcium are correlated with hypoxic injury in cultured astrocytes. Some of the proposed therapies for stroke based on the hypothesis that limiting calcium influx into the astrocytes may also have a beneficial effect upon astrocyte survival.


A precipitous depolarization during ischemia and hypoxia marks the severe compromise of membrane potential in widely scattered neuronal populations. It has been suggested that the latency to onset of hypoxic depolarization (HD) is a critical determinant of the fate of a neuron. In support of this concept, vulnerable CA1 neurons but not relatively resistant dentate gyrus granule cells (Balestrino et al., Brain Res. 497, 1989) and treatments that prolong the onset of HD alter pathophysiology. We examined whether variations in HD latency characterize the responses of differentially vulnerable hippocampal pyramidal cells.

In vitro hippocampal slices were prepared from 10-15 day-old rats in each experiment, simultaneous recordings were made in two of the following regions: CA1a, CA1b and CA3. Slices were maintained at interface with the recording chamber and hypoxic treatment consisted of substituting 95% N2 for the normal 95% O2 in the atmosphere. To verify viability of the slices, evoked synaptic responses were tested prior to and following the hypoxic insult. The response of CA1b was virtually identical in areas CA3 and CA1b. Experiments in which DC potentials were monitored simultaneously in these two regions showed that one area usually underwent HD slightly before the other with CA3 being first in 46% of the cases and CA1b first in 54% of the cases (n=50 slices). The relative, regional latencies to HD (CA1b<CA3<CA1a) did not parallel the relative vulnerabilities of these regions (CA1a<CA1b<CA3), from most to least sensitive. It therefore appears that regional variations in the latency to HD do not explain the differential vulnerability of subpopulations of hippocampal pyramidal cells.

Supported by NINDS grant NS24762.

502.3 MEASUREMENT OF INTRACELLULAR CALCIUM IN RAT CEREBRAL CORTICAL TISSUE DURING HYPOXIA AND AGLYCEMIA. Keith J. Brooks & Philip I. Aragon*, Departments of Pharmacology and Medicine, UMDS, St Thomas's Campus, London SE1 7EH; U.K.

Intracellular Ca ([Ca]i) has been implicated as a probable mediator of neuronal damage during cerebral ischemia. In order to study the effects of hypoxia on [Ca]i, awake ferrets were anesthetized and loaded cortical slices (350 μm thickness) with fura 2 AM (10 μM) or 1,2-bis (2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM) for 1 hour at room temperature. Tissues were subsequently superfused with Krebs solution, gassed with 95% O2, 5% CO2, at 37 °C. Tissues were judged if de-differentiated with 40 mM K solution caused a substantial increase in in [Ca]. When glucose was removed from the solution, there was a delayed rise in [Ca]; if hypoxia was induced by gassing with N2 instead of O2, there was little change in [Ca]. If, however, tissues were made both hypoxic and aglycemic, [Ca], rose rapidly and markedly. This result suggests that it is necessary to induce both these insults simultaneously in order to cause immediate disruption of the [Ca], regulating mechanisms in cortical tissue.


Excitatory amino acid transmission has been demonstrated to contribute to the neuronal injury that occurs as a result of hypoxic insults. We studied the contribution of alterations in EAA transmission on the resting leak current measured from CA1 pyramidal neurons during hypoxia. In vivo hypoxic slices were made hypoxic by exposing them to a gas mixture containing 95% N2/5% CO2. Whole-cell patch-clamp recordings were made from CA1 neurons with K-glucuronate (W=60 mV) in control neurons (n=11). Hypoxia resulted in the development of a slowly increasing inward current within 60 sec of exposure to nitrogen gas. This current was interrupted by a rapidly developing, high-amplitude (RIC) (mean latency=157+/-35 sec), which was typically associated with a negative shift in the field potential. Selective inhibition of non-NMDA glutamate receptors with 5 μM CNQX resulted in a moderate increase in the latency of the RIC (n=10, 522+/-39 sec), as did blocking propagated synaptic transmission with 1 μM TTX (n=5, 391+/-49 sec) or cutting the Schaffer collaterals (n=7, 485+/-48 sec). Selective inhibition of NMDA receptors with 5 μM CPP (n=8) or non-selective blockade of both NMDA and non-NMDA glutamate receptors with 5 μM kynurenic acid (n=5) resulted in a more marked increase in the latency of the RIC (792+/-135 sec and 787+/-118 sec, respectively). These data are consistent with previous studies in which hypoxia was evaluated with extracellular and current clamp techniques. In addition, they suggest that both non-NMDA- and NMDA-mediated events may be involved in hypoxia-induced cell injury. (Supported by NINDS grant NS1460-01).

502.5 Effects Of Hypoxia On Spontaneous And Evoked Synaptic Currents In The Hippocampus. B. Herabkova and S. Vergeer, Dept. of Neurology, Georgetown Univ., Wash, DC 20007.

Little is known about the influence of hypoxia on synaptic transmission. We examined the effects of hypoxia on synaptic activity in CA1 neurons of the hippocampal slice using patch-clamp techniques. A stimulating electrode placed in the stratum radiatum was used to elicit orthodromic synaptic responses. Slices were exposed to hypoxia by exposing them to a gas mixture containing 95% N2/5% CO2. Orthodromic stimulation elicited an inward current followed by an outward current (IPC). In addition to evoked currents, small spontaneous inward currents were also observed. Hypoxia resulted in a gradual decline in the evoked EPSC amplitude and a rapid decline in the evoked IPC amplitude. Thus, after approximately 60 sec the IPC was 15% of control whereas the EPSC was 86 ± 7% of control (n=5). A pure NMDA channel could be elicited in two experiments carried out in the presence of CNQX (5 μM). Under these conditions, the orthodromically elicited inward current was rapidly inhibited with complete suppression occurring within 90 sec of hypoxia also resulting in an increased frequency of spontaneous inward currents in many of the cells examined. Cutting the Schaffer collaterals did not abolish this increase, but kynurenic acid (5 μM, n=5) and TTX (1 μM, n=5) suppressed these spontaneous currents, identified as NMDA-EPSC. These results suggest that glutamate release during hypoxia may play an important role in the development of hypoxia-induced seizures. (Supported by NINDS grant NS01460-01).

502.6 METABOLIC ADAPTATIONS TO ANOXIA IN THE ISOLATED TURTLE CEREBELLMUM. M. Perez-Pinero, J. Bedford, M. Rosenthal, P. L. Lutz and T. J. Sisk, University of Miami Schools of Medicine and Marine and Atmospheric Sciences, Miami, Fl.

Turtle brain can survive prolonged anoxia during which ion homeostasis (K+) is maintained, evoked potential activity is depressed but not suppressed, and ATP levels are unchanged from control values. Metabolic adaptation to anoxia tolerance likely include decreased ATP demand and increased anaerobic ATP production. Present goals were to account more precisely for such metabolic activity and to seek mechanisms controlling anaerobiosis during and subsequent to the transition to anoxia. In situ studies in an isolated turtle cerebellum which demonstrates similar anoxia tolerance to that of the turtle brain in situ. Unlike in mammalian brain or mitochondria which are O2 regulators (i.e. O2 consumption is independent of O2 availability over a broad range), O2 consumption in turtle cerebellum varied sensitively with O2 availability over the broad range), O2 consumption in turtle cerebellum varied sensitively with O2 availability over the broad range). These results indicate that changes in O2 levels during and subsequent to the transition to anoxia. Calculations from O2 consumption and heat output in turtle cerebellum by flow-through microcalorimetry demonstrated that ATP production decreased approx 50% during 2 hours of anoxia. Calculations from O2 consumption and heat output in turtle cerebellum by flow-through microcalorimetry demonstrated that ATP production decreased approx 50% during 2 hours of anoxia. Calculations from O2 consumption and heat output in turtle cerebellum by flow-through microcalorimetry demonstrated that ATP production decreased approx 50% during 2 hours of anoxia. Calculations from O2 consumption and heat output in turtle cerebellum by flow-through microcalorimetry demonstrated that ATP production decreased approx 50% during 2 hours of anoxia. Calculations from O2 consumption and heat output in turtle cerebellum by flow-through microcalorimetry demonstrated that ATP production decreased approx 50% during 2 hours of anoxia.
502.7 ELECTROPHYSIOLOGICAL ANALYSIS OF POST-ISCHEMIC CA1 NEURONS USING IN VITRO HIPPOCAMPAL SLICES. K. F. Cregan, K. W. Harris, Palmer Department of Physiology, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada.

Transient forebrain ischemia causes a loss of neurons in hippocampal area CA1 after a delay of 1-3 days. A leading candidate mechanism is the "excitotoxic hypothesis". The excitatory afferent hyperactivity, which develops after ischemia, causes CA1 cell death. We have examined synaptic transmission along the major excitatory afferent to CA1 (the commissural/associational, or C/A, pathway) at different times after transient ischemia in rats (similar studies have been carried out in gerbil; Urban et al., Stroke 21: 11-23, 1989).

Transient forebrain ischemia was induced using the "4-Vessel Occlusion" (4V0) model (Pulsinelli & Brierly, 1988). This procedure caused a marked reduction in blood flow in the anterior cerebral arteries (ACA), posterior cerebral arteries (PCA), and anterior communicating arteries (ACM). The ischemic period was 20 min and was followed by reperfusion for 10 min.

In the CA1 region of the hippocampus, the CA1 pyramidal cells were identified using an antibody to CA2+ ions. These cells were then imaged with a confocal laser scanning microscope to determine the degree of CA2+ ion accumulation.

The results showed that the CA1 pyramidal cells accumulated a significant amount of CA2+ ions, with the highest levels observed in the CA1 region. This finding is consistent with the "excitotoxic hypothesis" and suggests that the accumulation of CA2+ ions is a key mechanism for CA1 cell death after transient ischemia.

502.8 MECHANISMS OF ANOXIA-INDUCED DEPOLARIZATION IN BRAINSTEM NEURONS: IN-VITRO INTRACELLULAR STUDIES. G. S. Fradet, A. G. Maupas, M. D. MacDuff, Marguerite Pichard Research Centre, McGill University, Montreal, Quebec, Canada.

Transient forebrain ischemia involves the CA1 region of the hippocampus and the CA1 pyramidal cells are known to undergo anoxia-induced depolarization (AID) after ischemic events. The mechanism of AID in CA1 neurons is not well understood.

We measured AID in CA1 neurons using intracellular microelectrodes. The CA1 region was isolated from the hippocampus and placed in an oxygenated ACSF solution. The CA1 neurons were then impaled with intracellular microelectrodes and the membrane potential was measured.

The results showed that AID was mediated by the opening of voltage-gated potassium channels (Kv). This opening was caused by the decrease in extracellular Na+ and Ca2+ concentrations, which led to a decrease in the electrochemical gradient for these ions. This decrease in the electrochemical gradient caused a reversal of the resting membrane potential, leading to an increase in the membrane conductance and a depolarization of the membrane potential.

The results suggest that AID is mediated by the opening of voltage-gated potassium channels (Kv) due to the decrease in extracellular Na+ and Ca2+ concentrations, which leads to an increase in the membrane conductance and a depolarization of the membrane potential. The depolarization is further exacerbated by the increase in extracellular K+ concentration, which reduces the potassium conductance and leads to an increase in the membrane potential. These results have important implications for the development of therapeutic strategies for stroke and trauma patients.

One possible mechanism of anoxia tolerance is the "channel arrest" hypothesis which states that ionic currents across the plasma membrane is reduced during an anoxic episode. The reduction of ion leakage would reduce demand on ATP utilizing ion pumps, thus preserving ATP in metabolically essential organelles. We used the turtle (Chrysemys picta) as an anoxia tolerant model system, as this species has been shown to survive anoxia for over 6 months. The transection of ion leakage channels in vivo, while cell input resistance and specific membrane resistivity were measured under control and anoxic conditions using intracellular recording in cortical slices incubated at 25°C. There were no significant changes in measured parameters indicating that ion leakage was not reduced. These results do not support channel arrest as a mechanism of energy conservation during short term anoxia (< 120 mins.).


During spreading depression (SD) and hypoxic SD-like depolarization, intercellular calcium concentration ([Ca\(^{2+}\)]\(_o\)) decreases precipitously, presumably by influx into cells. The mechanism of the influx is not known. We used ion selective microelectrode techniques to test the effect, in the CA1 region of the rat hippocampus, of short blocking periods of voltage clamping on channels on the drop of [Ca\(^{2+}\)]\(_o\), as well as on the change in extra-cellular potential (V\(_e\)) and [K\(^+\)]. SD was induced by application of 1-2 μl 3M KCl solution to the brain surface in a 3M KCl solution or by withdrawal of the K\(^+\)-rich dialysis fluid 3 minutes prior to the SD. When Ca\(^{2+}\)-free and Ni\(^{2+}\)-free media were added immediately after the K\(^+\)-withdrawal, the decrease of [Ca\(^{2+}\)]\(_o\) and the negative shift of V\(_e\) were curtailed but not abolished, more so in st. pyramidale than in st. radiatum. For example, in K\(^+\)-induced SD the average effect of Ca\(^{2+}\)-free and Ni\(^{2+}\)-free media was to reduce [Ca\(^{2+}\)]\(_o\) to 72% of control value in st. pyramidale and to 94% of control value in st. radiatum. At the site of high K\(^+\)-application SD-related changes were only moderately depressed by Ca\(^{2+}\)-free or Ni\(^{2+}\)-free media at some distance from the focus of propagation of SD was powerfully suppressed. We conclude that (1) During SD some but not all Ca\(^{2+}\) enters cells through voltage-gated channels; (2) SD-related membrane processes in neuron somata can vary independently from those in the dendrites; (3) The mechanism of SD initiation may be different from that of SD propagation. (Supported by NS 18670, 17771, 06233.)


During hypoxia of CNS synaptic transmission fails rapidly and, initially, reversibly. We tested the hypothesis that voltage-gated calcium channels in the presynaptic terminals are inactivated by hypoxia, leading to failure of transmitter release. Rat hippocampal slices were studied in a dual-well interface chamber at 34.5°C. Intestinal calcium concentration ([Ca\(^{2+}\)]\(_i\)) was recorded with ion-selective microelectrodes in st. radiatum of CA1 and sometimes also in st. pyramidale. Ortho- and antidromic stimulus trains (5s, 20Hz) were applied to evoke changes in [Ca\(^{2+}\)]\(_o\). In order to record [Ca\(^{2+}\)]\(_i\), responses due to Ca influx into presynaptic axon terminals, postsynaptic responses were blocked by 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 20 μM (±)-2-cyclohexapiparazine-4-yi-propyl-1-phosphonic acid (CPP); and, in other experiments, by lowering both Ca\(^{2+}\) to 0.2 ± 0.12 mM. Oxygen was withdrawn until signs of spreading depression (SD)-like depolarization occurred. During hypoxia orthodromic (presynaptic) [Ca\(^{2+}\)]\(_i\), responses in st. radiatum were extinguished before SD began; antidromically evoked [Ca\(^{2+}\)]\(_i\), responses in st. pyramidal failed only at the moment of SD. "Resting" baseline of [Ca\(^{2+}\)]\(_i\), declined first gradually, then, with the onset of SD, precipitously. In the presence of DNQX and CPP hypoxic SD occurred later, and during SD [Ca\(^{2+}\)]\(_i\) decreased less than in slices bathed in control solution. The findings are consistent with a failure of voltage-gated Ca\(^{2+}\) channels in presynaptic terminals during hypoxia, possibly due to inactivation caused by a rise of [Ca\(^{2+}\)]\(_o\). During hypoxic SD some but not all the Ca\(^{2+}\) appears to enter cells through glutamate-controlled channels. (Supported by grants NS 16675 and NS 06233.)

502.17 Hypoglycemic injury in organotypic hippocampal culture is similar to NMDA neurotoxicity and is prevented by delayed MK-801.  R. C. Tasker, I. J. Voreux and J. T. Cotyle Dept. Anesthesiology, Neurology, Neuroscience and Psychiatry, The Johns Hopkins School of Medicine, Baltimore, MD 21205

In animal models of hypoglycemia, neurotoxicity is rapid and selectively involves CA1 and the dentate gyrus. Injury is attenuated by concomitant NMDA receptor antagonists. In organotypic hippocampal culture, which preserves the regional differentiation of the hippocampus, we have examined the time course of neuronal injury following in vitro "hypoglycemia" using propidium iodide fluorescence microscopy to observe directly the regional distribution of membrane injury in real time. Increasing duration of exposure to glucose-free media, for up to 3 hours, was associated with an increasing severity of injury. The pattern of propidium iodide staining following 2 hours of hypoglycemia was the same as that observed following a 30 minute exposure to 10 μM NMAD: simultaneous staining of CA1 and the dentate gyrus and a negative shift of Vo were seen. The negative shift of Vo was curtailed but not abolished, more so in st. pyramidale than in st. radiatum. For example, in K\(^+\)-induced hypoxic SD the average effect of Ca\(^{2+}\)-free and Ni\(^{2+}\)-free media was to reduce [Ca\(^{2+}\)]\(_o\) to 72% of control value in st. pyramidale and to 94% of control value in st. radiatum. At the site of high K\(^+\)-application SD-related changes were only moderately depressed by Ca\(^{2+}\)-free or Ni\(^{2+}\)-free media at some distance from the focus of propagation of SD was powerfully suppressed. We conclude that (1) During SD some but not all Ca\(^{2+}\) enters cells through voltage-gated channels; (2) SD-related membrane processes in neuron somata can vary independently from those in the dendrites; (3) The mechanism of SD initiation may be different from that of SD propagation. (Supported by NS 18670, 17771, 06233.)

502.18 EFFECTS OF ANOXIA ON EXCITATORY AMINO ACIDS IN BRAIN SLICES OF RATS AND TURTLES: IN VITRO MICRODIALYSIS STUDY. R.B.K. Young, M.J. During, G.G. Hadadd, D.F. Donnelly, W.J. Aquila, V. Parisi. Yale University School of Medicine, New Haven, CT.

The purpose of this study was to utilize in vitro microdialysis to test the hypothesis that anoxia has a differential effect upon the concentrations of excitatory and inhibitory amino acids released from brain slices of rats compared to those of turtles. Ten minutes of anoxia (95% N\(_2\) - 5% CO\(_2\)) produced significant elevation of glutamate (from 0.39±0.03 to 0.90±0.18 μM, p < 0.05, ANOVA) in rat brain slices, whereas in turtle slices a decrease in glutamate (from 0.50±0.11 to 0.33±0.09, p < 0.05) was observed. We speculate that the mechanism of anoxia resistance in the turtle is at least partly due to the lack of increase in excitatory amino acids in response to severe oxygen limitation.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
503.1 DIFFERENT MODES OF HIV-1 TRANSCRIPTION ACTIVATION IN GLIAL, AND IN PHORBOL ESTER (PMA)- AND RETINOIC ACID (RA)-TREATED HUMAN NEURONAL CELLS; D. Mondal* and O. Prakash. Dept. of Molecular Oncology, Alton Ochsner Medical Foundation, New Orleans, LA 70121.

A variety of neurological dysfunctions, collectively known as AIDS dementia complex, are observed in AIDS patients. Previously, it was considered that macrophages or microglial cells were the only cell types which carried the virus in the brain. However, recently it has been shown that neurons can also infect neurons and glial cells. We carried out transient transfection experiments in order to assess the transcriptional activity of HIV-LTR in neuronal and glial cell lines.

HIV LTR expression was significantly higher in RA-treated compared to PMA-treated HIV-LTR reporter HIV-LTR cells. The NF-κB responsive element did not appear to play a major role in this activation. In addition, the LTR expression was refractory to tat-mediated transactivation and induction by TNF-α, insulin and morphine. On the other hand, HIV-LTR expression in PMA-treated cells was inducible with tat, TNF-α, insulin and morphine. In the astrocytoma cell line U-373 MG, the LTR expression was inducible with TNF-α and tat but not with insulin and morphine. These results suggest that neuronal and glial cells have the capacity to induce cellular factors which can activate HIV expression and can contribute to neurological dysfunctions. Studies are underway in our laboratory to characterize these factors.

503.2 RECOMBINANT G P120 CAUSES ASTROCYTE ALTERATION L. Pulliam, D. West*, N. Hargwoode*; R. A. Swanson Depts. of Laboratory Medicine and Neurology, VA Medical Center and Univ of CA, San Francisco, 94121 and Chiron Corp. Emeryville, CA.

The gp120 of HIV has been reported to be neurotoxic to rat neurons; however, these results were not consistent with pathologic changes observed in patients with AIDS. In order to further characterize this AIDS dementia and to define the role of gp120, we studied the effects of 2 recombinant gp120 preparations (SF2 and 3B) and gp120 SF2 deletion mutants on astrocytes in vitro. The 4 glycosylated recombinant gp120 molecules were used at concentrations of 1 pM to 1nM. The SF2 molecule binds to a CD4 receptor and 3B molecule does not. Using flow cytometry, we did not see a significant decrease in the neuron population nor was gp120 binding observed; however, there was a disruption of cellular matrix by histological examination. Using flow cytometry, there was a decrease in GFAP-staining cells treated with SF2 in 2 out of 4 brain preparations. By ultrastructure examination, cells consistent with astrocyte morphology but lacking cytoplasmic fibrils could be seen. To explore the possibility that altered astrocyte function may influence neuron viability, we measured GLUT-4 uptake of gp120 treated human astrocyte cultures and did not find a significant decrease.


We earlier used various antibody probes to characterize the classes of neurons in the V1 division of the mouse trigeminal ganglion (TG) that become infected with HSV-1, using Western and HRP methods. We have now examined the TG of HSV-infected mice bearing specific herpes simplex virus (Type 1) (HSV1) lesions or CMV lesions (bilateral V1). In the V1-lesioned TG, the spared neurons were primarily those innervating the cornea, dependent on their location on the cornea and the distribution of the interneurons, the corneal sensory neurons, and the distal dendrites of the corneal afferents. Approximately 90% of the neurons were infected in the TG of HSV-infected mice bearing HSV-1 lesions. In the V1-lesioned TG, the spared neurons were primarily those innervating the cornea, dependent on their location on the cornea and the distribution of the interneurons, the corneal sensory neurons, and the distal dendrites of the corneal afferents. Approximately 90% of the neurons were infected in the TG of HSV-infected mice bearing HSV-1 lesions.


Tau proteins are low molecular weight microtubule-associated proteins found in the protease-resistant paired helical filaments of neurofibrillary tangles in neurons in Alzheimer disease (AD). Approximately 66% of all AIDS patients suffer from clinical dementia. The loss of cognitive function in AIDS is similar to that in Alzheimer disease. The loss of cognitive function in AIDS is similar to that in Alzheimer disease. The loss of cognitive function in AIDS is similar to that in Alzheimer disease.

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503.5
S100β IS ELEVATED IN BRAIN CELLS OF AIDS PATIENTS.

We have recently reported an elevation of IL-1 in microglia in AIDS temporal lobe because IL-1 has been shown to stimulate astroglisis in rats (Guilian et al., 1988). We proposed that elevated IL-1 induces astroglisis, including synthesis of S100β in Alzheimer Disease and Down Syndrome, and neurodegenerative disorders, including AIDS, where glosis is a characteristic neuropathology (Griffin et al., 1989). We have recently shown an elevation of the level and neuropathic activity of S100β in AD and suggested that this elevation contributes to the hyperactivity of neurons in senile plaques (Marshak et al., 1991). In order to determine if reactive astrocytes, containing elevated levels of S100β, are present in AIDS temporal lobe along with the elevated levels of microglia-derived IL-1, formalin-fixed, paraffin-embedded temporal lobe sections from HIV infected individuals (AIDS, n=6), are age-matched controls (AMC, n=6) were examined, using a specific antibody to S100β (gift from D. Marshak). The number of S100β immunoreactive astrocytes in AIDS was approximately 2 times that in AMC in both grey and white matter. Furthermore, these cells were enlarged and had prominent processes. We conclude that astroglisis with elevated expression of S100β is associated with HIV infection and elevation of microglia-derived IL-1 in AIDS. If this elevation of S100β in AIDS is reflective of neurotrophic activity, as in AD, it could lead to the formation of dystrophic neurites and neuronal dysfunction. This work was supported in part by NS27414.

503.7
IDENTIFICATION OF UBIQUITIN-CONTAINING CELLS IN THE BRAIN FROM AIDS PATIENTS. H. Asari and F. J. Dansky, Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Ubiquitin belongs to a class of proteins produced by cells in response to physiological stress. Of central importance is ubiquitin's role in the proteolytic degradation of ubiquitin-protein complexes. These complexes are a signal for the degradation of both normal and abnormal proteins. To date, ubiquitin has been identified in lymphoid, myeloid, mesenchymal, and ciliated epithelial cells. Therefore, ubiquitin is involved in cellular inclusions in tissues. The brain, therefore, is capable of producing ubiquitin and this production appears in association with various neuropathological states. Neurological problems and neuropathological lesions are associated with HIV infection. Work has been undertaken to clarify the role of ubiquitin and ubiquitin-like proteins in the production of the neurodegeneration which is found in AIDS. In the present study, we examined the presence of ubiquitin in the brain from AIDS patients (AIDS, n=6). We have been able to identify ubiquitin-like proteins in neurons and astrocytes in these brains. Immunohistochemical staining for ubiquitin in these brains is not due to a receptor unique to these cells. We determined the number of ubiquitin-immunoreactive cells in AIDS and controls. Additionally, we determined the number of ubiquitin-immunoreactive cells in response to HIV infection. We concluded that elevated expression of brain-derived IL-1β is associated with HIV infection and possibly contributes to the neuropathogenesis of dementia in AIDS. This work was supported in part by NS27414.

503.9
PCR AMPLIFICATION OF BORNA DISEASE VIRUS (BDV) SPECIFIC SEQUENCES IN NON NEURAL TISSUES AND BODY FLUIDS IN NEWBORN, CHRONIC AND ACUTELY-INFECTED RATS. A. M. Sierra-Honigman*, T. W. Lipkin, A. M. Sierra-Honigman, R. W. Maltsi, J. R. H. Volken*, and K. M. Carbon, Divisions of Infectious Diseases, Dept. of Pediatrics and Medicine, Dept. of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21285.

Borna disease virus (BDV) is an RNA virus which causes immunopathological neurobehavioral disease. We examined the preferential replication of BDV in neural cells, as compared to the MDCK cell line, a canine kidney cell line. BDV-infected cells were extremely permissive to BDV replication, as 100% of the cells expressed viral antigens after BDV inoculation. BDV-infected C6 cells, in contrast, were less permissive to BDV replication, as 100% of the cells expressed viral antigens after BDV inoculation. BDV-infected C6 cells were more permissive to BDV replication, as all of the cells expressed viral antigens after BDV inoculation. BDV-infected C6 cells produced more viral RNA and protein than the BDV-infected MDCK cells. BDV-infected C6 cells produced more viral RNA and protein than the BDV-infected MDCK cells. Therefore, the neurotropism of BDV may be associated with the production of soluble factors by neural cells.

503.10
NUCLEIC ACID SEQUENCE FOR THE ORF ENCODING THE 38 KD PROTEIN OF THE BORNA DISEASE AGENT. Gus Ray*, W. Jan Lipkin, Neurology; Anatomy & Neurobiology; Microbiology & Molecular Genetics, University of California, Irvine, CA 92717.

Borna disease agent (BDA) is an RNA virus characterized by tropism for pyramidal neurons of hippocampus and a wide vertebrate host range. Serologic data suggest that BDA or a related virus may cause neuropsychiatric disease in man. BDA RNAs are transcribed in the nucleus and then transported to the cytoplasm. Histochemistry indicates that BDA antigens are primarily found in neuronal location. Here we report nucleic acid sequence for the ORF encoding the major immunogenic protein of BDA. Predicted amino sequence for this 38 kd protein includes a putative nuclear import signal motif.
503.12 NEUROACTIVE KYNURENINES AFTER INTRASPINAL INOCULATION OF MACAQUES WITH LIVE POLIOVIRUS
P.M. Heyer*, K. Saine†, S.P. Markey* and J.H. Vickers*
Section on Analytical Biochemistry, NIMH and Pathobiology and Primate Laboratory, FDA, Bethesda MD 20892.

Immune stimulation increases the concentrations of quinolinic acid (QUIN), an excitotoxic kyurenine pathway metabolite (J. Neurochem. 31, 1946, 1988; Ann Neurol. 29, 202, 1991). In the present study, 66 rhesus macaques received an intraspinal injection of poliovirus (PV) as part of a neurovirulence test for vaccine safety and compared to 6 sham and 6 unvaccinated controls. Serum QUIN concentrations increased between 3- to 550-fold on day 17 and were highly correlated (r > 0.70) to increases in indeoleamine-2,3-dioxynoguan (IDO) activity and QUIN levels in the spinal cord. CSP QUIN, quinolinic acid and kyurenine acid levels were also increased and correlated with CSF QUIN levels and spinal cord IDO activity (r > 0.70). Smaller increases in IDO activity and QUIN levels were found in SCF (r < 0.70) with no changes in the frontotemporal cortex. The largest metabolic and histopathologic changes occurred in macaques with lower limb paralysis. Quantitative measures of neurologic damage and inflammatory responses in the spinal cord correlated with the magnitude of each of these metabolic responses in CSF and spinal cord (r > 0.78). Serum QUIN and L-KYN levels were unchanged in the PV-inoculated macaques. We conclude that PV-inoculated macaques offer a primate model to determine the effects of intracisternal immune stimulation on kyurenine pathway metabolism and to investigate whether endogenous production of neuroactive kyurenines in vivo are associated with functional or neuropathologic consequences.

504.1 REDUCTION IN GLUCOSE LEVELS ENHANCES 2'ET-MPTP-INDUCED TOXICITY IN PC12 CELLS

The active agent in MPTP neurotoxicity is thought to be its metabolic product, MPP+. MPP+ and analogs such as 2'ET-MPTP are actively accumulated by mitochondrial preparations in vitro and block NADH dehydrogenase of complex I. This blockade results in biochemical sequelae which are cytotoxic to a marked hyperpolarization of the plasma membrane, generalized endocrinopathy and marked hypoglycaemia-hypersinaemia. The extremely low toxicities noted in pancreas and other endocrine organs in 139H infected hamsters. In the current study, female, weanling, Syrian hamsters, strain LVG/LAK, were inoculated subcutaneously in the right inguinal region with 10^6 plaque forming units of PRV-Ba. The animals were sacrificed 21 days post-inoculation (dpi) and the pancreas and other endocrine organs collected for histologic study.

Previous studies showed that the 139H strain of scrapie infected intracerebrally in hamsters caused no noticeable changes in their eyes (J. Shek2.1 CSI/IBR Center for Developmental Neuroscience, Stanford University, Stanford, Ca 94305). The extremely low toxicities noted in pancreas and other endocrine organs in 139H infected hamsters. In the current study, female, weanling, Syrian hamsters, strain LVG/LAK, were injected intracerebrally with scrapie strain 139H or with normal hamster brain. Coronal sections of the pituitaries were stained with hematoxylin and eosin, and were examined by light microscopy. The scrapie strain 139H infected hamsters showed extensive vacuolization in the pancreatic islets. While vacuoles were located on the central and lateral parts of the parietal cells, the vacuoles were more abundant in the islets of Langerhans of the pancreas, generalized endocrinopathy and marked hypoglycaemia-hypersinaemia. The extremely low toxicities noted in pancreas and other endocrine organs in 139H infected hamsters.

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Society for Neuroscience Abstracts, Volume 17, 1991
M04.9
Species-specific action of gangliosides on MPP+ toxicity in vitro, E.J. Roisen, J. Sonnogård, G. Yorke and M. Gupta. Dept. of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

Previously, we have shown that MPP+ has a dose-dependent effect on the survival of PC12 cells and that the neuroprotection of these cells with a mixture of bovine basic gangliosides (BBG) can partially prevent MPP+ toxicity. In these studies we examined the efficacy of individual gangliosides in the mixture to reduce MPP+ cytotoxicity. PC12 cells were maintained in RPMI 1640 medium supplemented with 10% horse serum and 5% fetal bovine serum in the presence of Nerve Growth Factor (NGF, 40 ng/ml) and the test agents for 6 h prior to incorporation of MPTP into the mitochondrial compartment. Alternatively, the cells were maintained in the medium supplemented with NGF and GM1 or GM3 (not present in BBG) for 4 days prior to MPTP exposure and continued for 4 days. Cytotoxicity was examined morphologically on the basis of neurite development, cell confluence and an index of dying cells. In the unmodified, GM1 increased MPTP cytotoxicity. In contrast, GM3 surprisingly reduced MPTP-mediated degeneration. Furthermore, in the presence of NGF and GM3, GM3 (but not GM1) had a significant effect in preventing MPTP toxicity. Similar results were obtained with differentiated neurons. The neurotrophic potential of GM3 and the action of released ganglioside oligosaccharides is being assessed. GM3 was more effective in potentiating NGF-mediated neuritogenesis than GM1 and in promoting neurotrophic activity in preventing MPP+ cytotoxicity on PC12 cells. Supported by NIH NS24524 and the Parkinson’s Disease Foundation.

M09.11

Complex I of the electron transport chain is one of the two main sites of oxygen reduction in the mitochondria (Fehér, 1985). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is toxic to the dopaminergic system by inhibiting the rotenone-sensitive site of Complex I in mammalian mitochondria. Recently, it was reported that the substantia nigra of Parkinson’s Disease (PD) patients has a relative deficit of Complex I (The redox potential may be related to the production of either endogenous or exogenous toxins). We, thus, assessed possible inhibitory effects of other DA related compounds on mitochondrial rotenone-sensitive Complex I activity in rat brain. Mitochondria were prepared according to the methods of Clark and Nickols (1970) and preincubated with various concentrations (0.1-10 µM) of dopamine (DA), 6-hydroxydopamine (6-OHDA), dopamine 3,4-dihydroxyphenylalanine (DOPAC), L-DOPA and 6-OHDA. All four compounds caused significant inhibition of Complex I activity except for the lowest concentrations of DA and 6-OHDA. Inhibition occurred in the following order: 6-OHDA > DOPAC > L-DOPA > DA. The DA receptor antagonist fluphenazine (FLU), also blocked the activity of Complex I. These preliminary results also suggest that the reported abnormalities in Complex I activity in the substantia nigra of PD might be related to inhibition by endogenous dopamine.

M04.10

It has been shown that the caudal (lateral) portions of the substantia nigra (SN) is more severely affected than the rostral (medial) SN in idiopathic Parkinson’s disease, resulting in an uneven motor dysfunction and loss in the striatum. We have recently shown that MPTP analog, 2’-CH3-MPTP, produces a significant decrease in the number of tyrosine hydroxylase (TH)-positive tyrosine hydroxylase-positive cells in the striatum. To further explore the neurotoxic effects of 2’-CH3-MPTP on TH-positive neurons in the SN and glialosis in the striatum. Young adult male C57BL/6/J mice were treated with 2’-CH3-MPTP (total dose 45-60 mg/kg, i.p.) over a two day period, anesthetized and perfused with the fixative three days after MPTP and adjacent 40 µm thick serial sections through the brain were immunostained for TH and Glial Fibrillary Acidic Protein (GFAP). The SN was divided into a medial and lateral compartment and the number of TH-positive neurons were quantitated. The number of GFAP-positive astrocytes were quantitated in medial, lateral, dorsal and ventral compartments of the striatum. The results show that 2’-CH3-MPTP produces a dose-dependent decrease of TH-positive neurons in both the compartments of SN and an increase in GFAP-immunoreactive astrocytes in the striatum. The following will be discussed: Is the reduction in TH-positive neurons more significant in any one of the compartments of SN. Is there a comparable effect on glialosis in the striatum. Supported by USPHS grant NS13226 to MG.
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The mechanism whereby methamphetamine causes the long-term depletion of striatal dopamine is unknown. However, there are parallels between methamphetamine- and ischemia-induced DA depletions. For example, both insults are accompanied by a large increase in extracellular DA, and the loss of tissue DA can be prevented by prior inhibition of DA synthesis. Thus, we hypothesized that other parallels might also exist and provide insights as to a shared mechanism for the neurotoxic effects. Ischemic insult is known to increase free arachidonic acid as well as other fatty acids in whole brain and in striatum. This is accompanied by an elevation in arachidonic acid metabolites, such as prostaglandins and lipoxigenase products, and some of these products may contribute to the neurotoxic process. This proposal is supported by the observation that inhibition of arachidonic acid metabolism attenuates ischemic brain damage.

To examine the role of arachidonic acid metabolism in methamphetamine-induced DA depletion, we examined the impact of pretreatment with an inhibitor of the cyclooxygenase component of prostaglandin synthesis, indomethacin. Methamphetamine (3.35 - 25.0 mg/kg, s.c) was administered to male Sprague-Dawley rats (290-330 g) every 2 h for a total of 4 injections. When striatal tissue was analyzed by HPLC for DA content 7 days later, methamphetamine had caused a dose-dependent decrease in DA levels with a maximal effect observed at 12.5 mg/kg. While the drug effect was examined over time, DA depletion was maximal by 5 days and constant for at least 14 days. Indomethacin (5 mg/kg, s.c.) was then given 1 h before and 4 h after methamphetamine (4.12.5 mg/kg) and DA levels were measured 1 week later. Methamphetamine alone caused a 57% depletion of DA (saline, 157.3 ± 4.6 ng/mg protein; methamphetamine, 66.8 ± 18.6 ng/mg protein). This effect was attenuated by indomethacin (methamphetamine ± indomethacin, 88.4 ± 13.6 ng/mg protein). Thus, our initial results do not support the hypothesis that methamphetamine acts to deplete striatal DA via the cyclooxygenase component of arachidonic acid metabolism.

505.5 PERSISTENT NEUROTOXIC EFFECTS OF METHAMPHETAMINE ON DEVELOPING DOPAMINE (DA) AND SEROTONIN (5-HT) NEURONS IN REAGGREGATE TISSUE CULTURE. L. Won, P.C. Hoffmann and A. Hallander, Departments of Pharmacology and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Methamphetamine (Meth) produces long-term reductions in brain DA and serotonin levels in adult animals (Picare et al., Brain Res. 193: 153-163, 1980). In order to study whether there is a similar persistence of Meth's effect on developing neurons, we have used reaggregate culture containing mesencephalic tegmentum and corpus striatum from embryonic mice. Reaggregates were exposed for an acute (4 hrs of treatment on day 22 of culture) or chronic period, between 15-22 days of culture) to 10-4 M Meth and then allowed to recover (7 days for acute; 20 days for chronic exposure) in drug-free media. During the recovery period, samples of drug-treated, as well as control reaggregates were collected for analysis of monoamine levels. At the end of the acute or chronic drug treatment period, DA and 5-HT levels in Meth-treated reaggregates were markedly depressed, and 5-HT levels were 39% and 64% of control, respectively, for the acute treatment; 28% and 15% of control for the chronic exposure). During the recovery period, DA and 5-HT levels increased in both acute and chronically-treated reaggregates, as well as in control cultures. Despite increases in the treated groups, neither DA or 5-HT attained the levels observed in control reaggregates by the end of the recovery period. As with adult neurons, it is clear that the effects of Meth on developing neurons are marked and persistent. Supported by MH46134 and NIDA#271-90-7404.

505.7 EFFECTS OF AMBIENT TEMPERATURE AND REPEATED INJECTIONS OF METHAMPHETAMINE ON CAUDATE MONOMINES AS MEASURED BY CEREBRAL MICRODIALYSIS. B. Gough, J. Bower, S.P. All, W. Skljer Jr. and B. Holson, Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Female Sprague-Dawley rats were injected four times at 2-hr intervals with vehicle or 5 mg/kg methamphetamine (METH). Some rats were caged individually at room temperature, while others were housed individually in cold room (4°C). Extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxy indole-acetic acid (5-HIAA), serotonin (5-HT) and homovanillic acid (HVA) were assayed in the caudate of these freely-moving rats using microdialysis and HPLC-EC. Dialysates were collected over 4 min intervals, beginning 24 hr following dosing. METH reduced the critical significance on caudate monoamines, DA and 5-HT release was enhanced, while extracellular levels of 5-HIAA, serotonin (5-HT) and homovanillic acid (HVA) were reduced. Cold depressed extracellular levels of DOPAC, 5-HIAA and HVA, with or without METH exposure. The inhibition of DA and 5-HT release by METH and cold in these metabolites was additive. This cold-induced depression of caudate monoamine metabolites may be related to the ability of METH to block toxic METH effects on caudate 5-HT and DA content.

505.4 CALCIUM CHANNEL BLOCKERS ATTENUATE SEROTONIN NEUROTOXICITY INDUCED BY (±)-3,4-METHYLENEDIOXY-METHAMPHETAMINE (MMDA). M. Martello, A. Martello and G. Ricciarelli, Dept. of Pharmacology, Faculty of Medicine, University Cattolica of the Sacred Heart, Via Traversa 31, 00153, Rome, Italy.

The finding that the N-methyl-D-aspartate (NMDA) receptor blocker, dextromethorphan, prevents the serotonin (5-HT)-selective effects of (±)-3,4-methylenedioxymethamphetamine (MMDA) in rats (Finnegan et al., Neurosci. Lett. 95: 300-304, 1989) suggests that excitatory amino acids (EAAs) in the neurotoxic action of MMDA. EAAs have been postulated to mediate neuronal injury by triggering excessive influx of calcium (Ca++) into cells. The purpose of the present study was to test the hypothesis that Ca++ is involved in the neurotoxic action of MMDA. Rats were treated with either saline alone and in combination with various doses of Ca++ channel blocking agents (flunarizine, nimodipine, verapamil). All of the Ca++ channel blockers examined afforded either partial or complete protection, depending on the dose of the blocker used and the regimen of MMDA administered. As none of the Ca++ channel blockers interfered with synapsosomal 5-HT uptake, it is unlikely that their neuroprotective effect is related to an interaction with the 5-HT transporter. These results suggest that Ca++ is involved in the neurotoxic action of MMDA.


Exposure to toxic doses of METH is often associated with decreased striatal levels of cytosine hydroxylyase and DA. These experiments were designed to determine post METH changes in the presynaptic regulation of DA release and uptake using superfused striatal slices from male rats. DA levels in the striatum contralateral to that used for in vitro release were determined to be 8% of saline control 1 day post, 38% of control 3 days post, and 78% of control 5 days post (control 5 days post, 52.2 ± 6.3 ng/mg) and 78% of control 5 days post (control 5 days post, 52.2 ± 6.3 ng/mg). There were no changes in either the 15 µM K+ and 1 µM METH-evoked [3H]DA release or the amount of [3H]DA accumulated in striatal slices of rats pretreated with METH compared to control at any time point. These results indicate that, although METH decreased striatal DA levels, terminals may not have been destroyed. A 30% decrease (relative to saline control) in the 100 µM GLU-evoked [3H]DA release (151 ng/mg) was observed in striatal slices 1 day post METH but not from 14 day post METH rats. Therefore, a transient down-regulation in GLU/MDDA receptors may occur 1 day or less post METH. Since environmental temperatures of 4°C during METH exposure did not prevent a decrease in GLU-evoked DA release from striatal slices 1 day post METH but significantly blocked striatal DA level, decreases 1 days post METH, the relationship between the initial changes in GLU-evoked DA release and METH toxicity is unclear.

505.8 PROTEIN SYNTHESIS INHIBITION BLOCKS THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE IN RATS. K.T. Finnegans and R. Kelar, Dept. of Psychiatry and Pharmacology, Univ. of Utah Sch. Med. and the Salt Lake City VAMC, Salt Lake City, UT 84148.

Programmed cell death is a striking feature of normal embryonic growth and development. The CNS is a particularly common site, with an estimated one half of all neurons present during embryogenesis dying before adulthood. The survival of neurons appears to depend upon the continuous secretion of various trophic factors by target tissues. Historically, neurotrophic factors have been thought by promoting neuronal survival by antagonizing pro-apoptotic signals.

These factors are now understood to act by preventing the synthesis of new ('killer') proteins. Based on these findings, we examined whether protein synthesis prevents neuronal death induced by trophic factor deprivation. Cell death during embryogenesis thus appears to be an active process, involving the synthesis of many other proteins. Recently, however, it has been shown that certain pro-apoptotic proteins might also be involved in the neuronal damaging effects of methamphetamine (METH) in the mature rodent CNS. Male CF-1 mice were injected with METH, either alone or in combination with the protein synthesis inhibitor cycloheximide or anisomycin, and then killed one week later. Neostriatal DA, DOPAC, and HVA were assayed in METH alone induced a 50-60% depletion of neostriatal DA and its metabolites. Pretreatment with either cycloheximide or anisomycin, however, completely blocked the neurotoxic effects of METH. In other experiments, METH exposure failed to alter either cannabinoid-induced sterotomy or the threshold for convulsions induced by N-methyl-D-aspartate. The latter findings suggest that the neuronal damaging effects of cycloheximide are unlikely to be explained by an antagonistic action at either DA or MDDA receptors. The findings suggest that programmed cell death during embryogenesis and METH-induced neuronal cell death in the mature CNS involve similar biochemical mechanisms.
505.9

A number of drugs with an amphetamine-like structure can produce long-term neurotoxic effects on serotonergic neurons in brain. It has been suggested that the weak monoamine oxidase (MAO) inhibitory properties of these amphetamines contribute to their neurotoxic effects. If so, then more complete inhibition of MAO might potentiate the neurotoxicity of m-AMPH. We examined this postulate by administering regular DA overflow and did not attenuate m-AMPH-induced injury to striatal neurons. This finding set: 1) that the large increase in striatal DA overflow is correlated with terminal damage and 2) that ME-801 might prevent this damage by reducing the amount of DA released.

505.10

Four injections of methamphetamine (m-AMPH, 4 mg/kg, sc, at 2 hr intervals) produce severe DA neurotoxicity in rats. This neurotoxicity was assessed using MK-801 (0.5 mg/kg, ip) injections against pargyline toxicity 15 min before each m-AMPH treatment, before each of the last two m-AMPH injections, or before the first m-AMPH injection only. Each schedule of ME-801 pretreatment attenuated both the striatal DA overflow and the striatal DA loss as measured one week later. In contrast, a single injection of ME-801 15 min before the fourth m-AMPH treatment produced only a modest decrease in total DA overflow and did not attenuate m-AMPH-induced injury to striatal neurons. These findings support the hypothesis that dopamine may act as a low potency neurotoxin.
GLIAL- AND NEURONAL- SPECIFIC K+ CHANNEL GENE EXPRESSION IN THE CNS OF THE MOUSE. L. A. Adams*, K. M. Houamed, and B. L. Tempel. Departments of Medicine and Pharmacology, Univ. of WA, 98195; and GRECC, Seattle Veterans Affairs Medical Center, Seattle, WA 98108.

Voltage-gated potassium (K+) channels are important modulators of membrane excitability in neurons and other electrically polarized cells. We have examined the cellular distribution of two different K+ channel genes in the central nervous system (CNS) of the mouse. These genes, designated MK1 and MK2, each direct the expression, in injected Xenopus oocytes, of delayed rectifier channels with slightly different kinetic properties. Their respective patterns of CNS expression were analyzed using the technique of in situ hybridization, in which oligonucleotide probes directed against each different gene were 3'-end-labeled with 35 S-ATP and applied to 20 micron frontal sections of normal adult male mouse brains, using standardized protocols. We find that MK1 and MK2 have markedly different patterns of expression. MK1 is expressed in particular subsets of neuronal cell bodies, notably in regions of the brain which are known to produce high frequency actions potentials, i.e. the auditory pathway, vestibular pathway, and motor pathways, and the hippocampus. In contrast, MK2 is expressed in the major fiber tracts of the brain, and thus appears to represent a glial cell-specific K+ channel gene.
510.3
Molecular Mechanisms of Ionic Selectivity Identified by Site-Directed Mutagenesis in the Shaker K+ Channel. Andrei Tsoo, Russ Vickers,* Robert Berget,* and Christopher N. Squire.* Molecular, Cellular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

To identify the region of the K+ channel that influences ionic selectivity, we used site-directed mutagenesis to charge single amino acids in the Shaker K+ channel from Drosophila. Our data support the hypothesis that the H5 region (asparagine 431 to the asparagine 441) lines the putative channel and that mutants in the H5 region could alter the size of the pore. Mutations at residues outside of H5 had little effect on selectivity. Many of the mutations created within H5 produced nulls, yielding no detectable currents when expressed as homomultimers. Preliminary results have demonstrated that some of these nulls can be rescued by pairing the mutant subunits with wildtype subunits in tandem constructs that link the two subunits into a single polypeptide. These hybrid channel constructs produce effects on selectivity that are consistent with the H5 hypothesis.

510.4

In the chimeric channel DRK-NGK (Hartmann, Science 1991), a stretch of cDNA encoding 21 amino acids was exchanged between the donor channel rat-NGK (Drew, Baylor Plaza, 77030 Houston, TX) and the host channel DRK1 (Frech, Nature 1989). The expression conferred upon the host channel the phenotype of the pore of the donor channel, rat-NGK2. Thus, DRK1 had the same receptive properties as the donor rat-NGK2 channel which was about three times that of the host DRK1 channel and, DRK-NGK like NGK2, was blocked by the open channel blocker TEA at an external site whereas DRK1 was not blocked with external application but with internal application. It follows that whereas DRK-NGK and DRK1 must have arisen from the nine amino acid differences exchanged between DRK1 and rat-NGK2. We reversed each of these nine amino acids using site specific mutagenesis and tested the results on conductance and TEA blockade. One reversion, L3704F, restored an exaggerated version of the conductance of K+ and Ba by DRK1 although the TEA blocking profile was unaffected. Two reversions, Q586R and M325F, reduced inward current and block by external TEA. Supported by National Institutes of Health Grant Nos. NS38805 to J.A. Drew, NS28407 to R.H. John, and HL32862, HL37044, HL60800 and NS24377 to A.M. Brown.

510.5

Chimeric K+ channels (DRK/NGK) formed by replacing the pore region of DRK1 with that of NGK2 were shown previously to have the TEA blocking profile of NGK2; extracellular TEA was much more potent than intracellular TEA. In the present study we tested a hydrophilic, TEA derivative, tetranyloctammonium (TPeA) in DRK/NGK and mutant chimeras in which single amino acids in the pore region were restored to the corresponding DRK1 residues. Macropore K+ currents were measured in Xenopus oocytes. External TPeA blocked DRK/NGK in a time- and voltage-dependent manner (EC50 = 88 uM). Based on its voltage dependence, TPeA is thought to have a large conductance channel and a TEA blocking channel. Consistent with this idea we found that TPeA sensitivity was unchanged in Q586R, a mutant with markedly reduced sensitivity to external TEA. In contrast, L374V, with a mutant ion selectivity (Drewe, et al., Nature 1991), was found to be less sensitive to TPeA. Of the two amino acids in the H5 region of DRK1, intracellular asparagine 431 and asparagine 441 were shown to be critical for the action of TPeA. N586R and N586K, both in the pore region of DRK1 with that of NGK2 were shown previously to have the slope conductance of the DRK1 channel. N586R and N586K were found to be hyperpolarization with a conductance of 75 pS. This is consistent with the idea that the H5 lines the conductive pathway; both of these mutations reduce bulkiness, and maintain or increase the polar nature of the side chain. Mutations at residues outside of H5 had little effect on selectivity. Many of the mutations created within H5 produced nulls, yielding no detectable currents when expressed as homomultimers. Preliminary results have demonstrated that some of these nulls can be rescued by pairing the mutant subunits with wildtype subunits in tandem constructs that link the two subunits into a single polypeptide. These hybrid channel constructs produce effects on selectivity that are consistent with the H5 hypothesis.

510.6
Gating currents from a chimeric K+ channel expressed in Xenopus oocytes. M. Tagliatela, G.R. Kirsch, A.M. Brown and J. Stefani. Dept. Molecular Physiology and Biophysics, Baylor College of Medicine, and Dept. Anesthesiology, Baylor College of Medicine, 1 Baylor Plaza, 77030 TX.

Structural rearrangements of the voltage sensor of voltage-gated ion channels under the influence of the electric field generate gating currents. We have studied the gating currents of a chimeric delayed rectifier (DRK) K+ channel, DRK/NGK M3, in which the pore phenotype of the donor NGK2 was exchanged for that of the host DRK1. A single point mutation (L374F) caused a dramatic increase in the single channel conductance of the chimeric channel. The experiments were performed with a novel open-occluded voltage-gate method. Many properties of the DRK/NGK M3 gating currents were similar to those reported for DRK and NGK, except for the effect of the squib giant axon. This is consistent with the idea that the H5 region lines the conductive pathway; both of these mutations reduce bulkiness, and maintain or increase the polar nature of the side chain. Mutations at residues outside of H5 had little effect on selectivity. Many of the mutations created within H5 produced nulls, yielding no detectable currents when expressed as homomultimers. Preliminary results have demonstrated that some of these nulls can be rescued by pairing the mutant subunits with wildtype subunits in tandem constructs that link the two subunits into a single polypeptide. These hybrid channel constructs produce effects on selectivity that are consistent with the H5 hypothesis.

510.7
Mutations in the leucine heptad repeat of Shaker 29-4 alter the channel's activation without altering gating currents. Nathan B. Schoppa,* Ken McCormack,* Mark A. Tatoosh,* and Fred J. Sigworth.* Dept. of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510, Dept. of Entomology, University of California, Berkeley, CA 94720.

Mutating either of the two leucines in the conserved heptad repeat region of Shaker 29-4 causes a 60-100 mV depolarizing shift and a dramatic reduction in the voltage sensitivity of channel activation (McCormack et al., PNAS 88, 5931). In search of an explanation for these changes, we recorded macroscopic ionic currents and gating currents in membrane patches from oocytes injected with mRNA encoding non-inactivating wild-type and mutant channels. All measurements were made in the inside-out configuration and all data were expressed in terms of the single channel conductance. Mutations were introduced to the leucine mutations in the wild-type and mutant channels. The number of effective channels displaced per patch was determined in the two channels. Lower bounds for z obtained from the limiting slopes of the activation curves (at P(0) < 0.01) were similar: 9.9±1.3 (N=4) and 8.7 (N=2) for wild-type and mutant channels. These values were close to those obtained from experiments which were directly evaluated z. First, the number of channels in a patch was estimated from noise analysis on ionic currents; then, following the replacement of K+ with TEA, the charge movement was measured in each patch. As s was calculated to be 8.9±1.8 (N=5) and 8.6±1.9 (N=5) for the wild-type and mutant channels. The mutations appear to affect steps in the activation process that follow most of the charge displacement.
Mouse photoreceptors express mShak family potassium channels.

D. J. Kandel, C. Bruchez, B. Song, and H. P. Pożoga. Department of Biochemistry, Molecular Biology and Cell Biology and the Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Mouse potassium channels can be classified into at least four families based on sequence homology with the potassium channel families of *Drosophila*. The most extensively characterized rodent potassium channel family, mShak, is expressed in the rodent retina. However, the diversity of expression of mShak channels within specific neuronal populations is not yet known. To determine the diversity of expression of mShak channels within a specific neuronal type, RNA PCR was used. Mouse retinas were enzymatically dissociated and individual photoreceptor cells were harvested with a broken patch pipette. Following cell lysis by detergent, mRNA was purified using bead-coupled oligo(dT). cDNA was then synthesized from purified photoreceptor mRNA. The polymerase chain reaction was used to amplify mShak sequences from photoreceptor cDNA and specific primers specific for conserved mShak regions. The predicted amplification product was detected by gel electrophoresis. A central reaction without reverse transcriptase generated no such amplification product. Cloning and DNA sequence analysis of the putative photoreceptor mShak amplification products revealed the presence of MK2 sequences due to homology with RCK2 (Grupé et al., EMBO J. 9: 1749-1756). Therefore, mouse photoreceptors express mMK2 channels. Supported by NIH R01 EY0126 and EY02536.

MOLECULAR CLONING OF A NEW K+ CHANNEL IN APLYSIA

B Zhao and E R Kandel

Ctr Neurobiol & Behav, Columbia Univ, & HHMI, N Y, N Y 10032

Potassium channels are the major targets for neurotransmedulation and thus contribute to neural plasticity. Since a large number of K+ channels exists, and different K+ channels play different roles in defining various aspects of the electrical properties of a neuron, we have tried to clone new families of K+ channels. Toward this end we have cloned at least one new K+ channel from Aplysia using a PCR cloning strategy.

We carried out PCR reactions with degenerate oligonucleotides that code for conserved regions of known K+ channels. The right-sized PCR products were cloned and sequenced. The predicted product of this K+ channel is identical to other known K+ channels in the Aplysia genome. This K+ channel appears to be expressed in two putative cell types. This type of sequence identity suggests that it is a putative K+ channel and represents a novel family of K+ channels.

Aplysia retina contains a large transient K+ current. This expressed current in *Aplysia* oocytes will be cloned using cDNA libraries and expressed in oocytes. Thus, it appears possible to generate a novel potassium channel that is simply a homomultimer. Moreover, by expressing the current at high level, we could redesign critical electrical properties of identified neurons. In the bursting cell R15, overexpression resulted in an increase of the afterhyperpolarization and a narrowing of the action potential which altered the bursting pattern in the cholinergic neuron L10. Overexpression and consequent spike narrowing decreased the amount of transmitter release from the terminals and greatly weakened the synaptic connections. In many cells, prolonged period of inhibition (10-30 hr after injection) induced the additional expression of a more slowly inactivating or non-inactivating component in the current that modified still further the electrical properties of neurons. For example, it silenced completely the bursting of cell R15. We are now investigating how this change in expression is brought about.

A BODY CENTRED COORDINATE SYSTEM IN THE POSTERIOR PARIETAL CORTEX

P. R. Brodsky and R. A. Andersen

Dept of Brain & Cognitive Sciences, Massachusetts Institute of Technology, MA 02139.

Models of gaze shifts suggest the existence in the brain of a visual map in body centred coordinates. A distributed map of visual targets in head centred coordinates has previously been proposed for areas 7a and LIP of posterior parietal cortex in the monkey (Andersen et al., J. Neuroscience 10(4), 1990) since cells in these areas have a retinal receptive field, modulated as a linear function of eye position (formed a gain field). Head position would need to be incorporated into the activity of these cells to produce a body centred map of the visual world. We recorded cells from areas 7a and LIP in a monkey with a stereotaxic frame to move horizontally. The animal was trained to orient its head and eyes towards independent locations and make visually guided saccades from different initial eye and head positions. This allowed us to determine sequence of activity during the visual saccade task caused by eye position and that caused by head position.

To cells from areas 7a and LIP were recorded. Both eye and head position signals were observed, often in the same cell so that the cell's activity was encoding gaze position (eye plus head position). These signals were found to linearly modulate the visual or saccadic activity of 53 cells to produce gain fields to eye and head position (P<0.05). In 22 cells modulation of the neuronal response occurred with eye position but not head position whereas modulation of neuronal activity by head position was seen in 33 of 33 cells. The gain fields for these 33 cells were best described as functions of eye position rather than just head position as virtually all cells with gain fields for head position had similar gain fields for eye position. In the vast majority of these cells the gain fields were oriented towards the contralateral side of the body. These results suggest that cells in areas 7a and LIP are of two types. One type of cell incorporates both eyes and head position and can thus participate in encoding the location of a target in head centred coordinates. The other type of cell modulates retinal response as a function of gaze position rather than eye position and is thus capable of participating in encoding the location of a target in body centred coordinates.

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10A.12 OVEREXPRESSION OF AN APLYSIA SHAKER K+ CHANNEL CHANGES THE ELECTRICAL PROPERTIES AND SYNAPTIC EFFICACY OF IDENTIFIED APLYSIA NEURONS: B Zhao & E R Kandel, HHMI, Columbia, NY.

To examine the properties of a cloned Aplysia K+ channel (AK01a) in its native neuronal environment, we co-expressed different identified Aplysia neurons a plasmid expression vector (pHEX, Kaang et al., this meeting) containing the AK01a nucleotide sequence (pHEX-AKO1a). As early as seven hours after microinjection of pHEX-AKO1a, we observed a large transient K+ current (I transient) in the biophysical and pharmacological properties of one of the native transient K+ currents (Iadap). A similar current was obtained when AK01a was expressed in *Xenopus* oocytes. Thus, it appears possible to generate a native current with a channel that is simply a homomultimer. Moreover, by expressing the current at high level, we could redesign critical electrical properties of identified neurons. In the bursting cell R15, overexpression resulted in an increase of the afterhyperpolarization and a narrowing of the action potential which altered the bursting pattern in the cholinergic neuron L10. Overexpression and consequent spike narrowing decreased the amount of transmitter release from the terminals and greatly weakened the synaptic connections. In many cells, prolonged period of inhibition (10-30 hr after injection) induced the additional expression of a more slowly inactivating or non-inactivating component in the current that modified still further the electrical properties of neurons. For example, it silenced completely the bursting of cell R15. We are now investigating how this change in expression is brought about.

A new potassium channel in its native neuronal environment, we co-expressed different identified Aplysia neurons a plasmid expression vector (pHEX, Kaang et al., this meeting) containing the AK01a nucleotide sequence (pHEX-AKO1a). As early as seven hours after microinjection of pHEX-AKO1a, we observed a large transient K+ current (I transient) in the biophysical and pharmacological properties of one of the native transient K+ currents (Iadap). A similar current was obtained when AK01a was expressed in *Xenopus* oocytes. Thus, it appears possible to generate a native current with a channel that is simply a homomultimer. Moreover, by expressing the current at high level, we could redesign critical electrical properties of identified neurons. In the bursting cell R15, overexpression resulted in an increase of the afterhyperpolarization and a narrowing of the action potential which altered the bursting pattern in the cholinergic neuron L10. Overexpression and consequent spike narrowing decreased the amount of transmitter release from the terminals and greatly weakened the synaptic connections. In many cells, prolonged period of inhibition (10-30 hr after injection) induced the additional expression of a more slowly inactivating or non-inactivating component in the current that modified still further the electrical properties of neurons. For example, it silenced completely the bursting of cell R15. We are now investigating how this change in expression is brought about.

ELECTRICAL MICROSTIMULATION Delineates 3 Distinct Eye-Move­­ment Related Areas in the Posterior Parietal Cortex of the Rhesus Monkey

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Previous work has subdivided the posterior parietal cortex (PPC) into distinct areas based on anatomical and recording results. We now demonstrate that this parcellation is also reflected in the effects of electrical microstimulation. Stimulation (500 Hz, 0.5 μA, 0.1 msec, train duration 100 msec, max. current 200 μA) was delivered in 2 monkeys while the animal’s eye movements were monitored. The areas are designated on physiological criteria since the animals are still being used in experiments. Area 7a: Whereas many sites proved to be unexcitable, stimulation of other sites elicited eye blinks (threshold: 50-100μA), which at several sites were accompanied by fast and wavy eye movements. The eye movements were directed to a circumscribed zone in the visual field directed saccades. Floor of IPS: Goal directed saccades into the contralateral hemifield were also elicited from the floor of the intraparietal sulcus (IPS). Unlike eye move­­ments elicited from area 7a, these saccades had straight trajectories. They were usually accompanied by brisk movements of the shoulders, pinnae and facial muscles (threshold: 25-50μA). Ipsilateral microstimulation elicited saccades (threshold: 25-50μA) which were rarely accompanied by non-eye movements. Although varying initial eye position affected both the amplitude and direction of the saccades, these saccades did not show convergence to a goal within the ocularmotor range. They were always directed into the contralateral hemifield. Some sites yielding upward component than downward component saccades. Our observations suggest that the function of area LIP is more confined to saccades than that of the other 2 areas.

THURSDAY PM POTASSIUM CHANNELS: MOLECULAR BIOLOGY II

12A1

A. C. ELEGANS POTASSIUM CHANNEL WITH HOMOLOGY TO DROSOPHILA SHAW. A. Wei, P. Jegla, and E. Salkoff.

Dept of Anatomy and Neurobiology, Albert Einstein College of Medicine, Bronx, NY.

Four voltage-gated potassium channel gene subfamilies defined by the Drosophila potassium channel genes, Shaker, Shal, Shab and Shaw are represented in a wide variety of metazoa, including mammals. We now report the isolation of a Shaw potassium channel gene homologue from the nematode A. elegans. A screen of genomic DNA was performed by PCR utilizing degenerate synthetic oligonucleotide coding for highly conserved regions of the four Drosophila potassium channel genes. The deduced amino acid sequence of the nematode channel closely resembles that of the fly Drosophila, having a common ancestor no later than six hundred million years ago, the deduced amino acid sequence of the nematode channel closely resembles that of the fly Drosophila, having a common ancestor no later than six hundred million years ago. The nematode channel closely resembles that of the fly Drosophila, having a common ancestor no later than six hundred million years ago. This suggests that the function of area LIP is more confined to saccades than that of the other 2 areas.
511.3 NEURONES IN THE MACAQUE LATERAL INTRAPARIETAL CORTEX (LIP) APPEAR TO ENCODE THE NEXT INTENDED SACCADE. T. L. Schall, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

LIP, a subdivision of intraparietal area 7, has been implicated in the planning of saccadic eye movements on anatomical and physiological grounds. In a delayed saccade paradigm, many LIP neurones maintain an elevated level of activity after the offset of the visual cue and until the saccade is made in that direction. We have interpreted this "memory" activity as reflecting the monkey's plan to make the next saccade. Here we present further evidence for this hypothesis.

1. When monkeys delayed saccades to auditory targets, some LIP neurones show memory activity. Generally, this memory activity is in the same direction as for visual delayed saccades.

2. Memory double saccade experiments: the two targets are flashed briefly, as in a "traditional" double saccade, but the fixation point remains on the first target for several hundred milliseconds after the second target is flashed. Only the effect of the first fixation point may make the saccades be made. For most LIP neurones, only sustained activity related to the first saccade was manifest in the memory period, even if the second saccade target appeared within the receptive field of the neurone.

3. Change of motor plan paradigm: in a variant of the delayed saccade task, we systematically varied the size and spacing of the visual targets, so that in some cases the second saccade appeared not to be part of the original plan. We found that the memory activity observed during the delay period was still present when the target was different from the one originally intended.

Thus it appears that activity in many LIP neurones reflects the monkey's plan to make the next saccade, regardless of how the saccade is evoked, whether it is part of a sequence, and whether indeed it is made.

511.5 NEURONS IN THE LATERAL INTRAPARIETAL AREA (LIP) OF THE MONKEY REMAP VISUAL SPACE IN CONJUNCTION WITH SACCADIC EYE MOVEMENTS. REMAPPING OF AN OPERATING MECHANISM. L. L. Colly, J.-R. Dubel, and M. E. Goldberg Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Visual neurones in LIP show evidence for dynamic remapping of visual space. They respond to stimuli outside the classically determined receptive field when the monkey makes a saccade that will bring the stimulus into it. We now show that for most LIP neurones dynamic remapping is a phenomenon which extends to the memory representation of a visual stimulus. We have tested neurones in a new paradigm in which the visual stimulus is briefly flashed (< 50 msec) outside of the receptive field and is followed by a target which elicits a saccade. The saccade brings the eye to a position where the stimulus was still present, would be in the receptive field. We have discovered that virtually all LIP neurones discharge in this task at a rate that is about half of their response to an actual visual stimulus. Control experiments show that this activity is not related to the eye movement but is instead related to the memory of the original stimulus. They respond to stimuli that the monkey would have seen in the receptive field if the saccade had been made.

511.6 AREA 7 AND THE SPONTANEOUS CAPTURE OF ATTENTION BY UNEXPECTED STIMULI. C. R. Olson, Department of Psychology, George Mason University, Fairfax, VA 22030.

Attention includes both top-down processes, in which an object is chosen voluntarily, and bottom-up processes, in which an object calls attention to itself. Area 7 is involved in top-down attention as indicated by the enhancement of neuronal visual responsiveness when a behaviorally irrelevant stimulus is presented just prior to an intended saccadic eye movement. We have also found that activity in LIP is increased when a stimulus is presented just prior to an intended saccade. Here we present further evidence for this hypothesis.

Single-neuron activity was recorded at 105 sites in Area 7 of two cats whose eye movements were monitored with surgically implanted chronic recording electrodes. The electrodes were reinforced with periodic food reward for remaining in a state of general alertness and for tolerating head restraint. No other aspect of their behavior was reinforced. Visual stimuli, presented in a visual field maintained by computer control, were presented for 500 msec. Neuronal activity was recorded for 2000 msec following target presentation. Enhancement of the visual response was not attributable to simple superposition of a motor burst on the visual burst because it occurred even on trials when the saccade was delayed by several hundred msec. I conclude that the strength of the neuronal response in Area 7 is a predictor of whether a stimulus will or will not capture attention.

511.7 MODULAR CONNECTIONS BETWEEN AREA V4 AND TEMPORAL LOBE AREA PITv IN MACAQUE MONKEYS. D.J. Fellem an and E. M cClendon, Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030.

Area V4 contains "modules" which receive input from either cytochrome oxidase thin- or inter-stripe compartments of V2 (DeYoe et al., '92; Zeki & Shipp, '93). Trazer injections into these V4 modules produce segregated labeling patterns in posterior IT (Van Essen et al., '91; and see DeYoe and Sisola, this meeting). In this study, we determine if projections from other visual areas to V4 are also segregated, particularly from visual areas that project to V2.

1. When fluorescent retrograde tracers were injected into V4, non-linear organization of label was observed in area PITv. Single, 150-250 nl injections of fluorescent dyes into POSTV produced a variable labeling pattern in V4. In most instances, label was segregated into clusters of different size, separated by undyed areas ranging in size from 2x2 to 5x5 mm with an average width of 4 mm and separation of 4-7 mm. In 2 cases, retrograde tracers were injected into separate loci in area PITv separated by 2-3 mm. In these cases, label was produced in separate interdigitating patterns of the two tracers. In other cases, labeled zones in V4 were larger and irregularly shaped and may reflect labeling of the same module at different loci.

2. The relationship of label in V4 to areas in the rest of the visual system is unknown. Within occipito-temporal cortex, cells labeled by each tracer were also segregated. In one case, cats in which V1 and V2 remained segregated when termining in V4, label from different tracers was separated by a linear ridge in the region extending from the lip of the V4 through the parahippocampalis gyrus. Area PITv, and possibly V3A and V4B, appeared to contain more than one patch of label. These patches were segregated by different functions. Within occipito-temporal cortex, cells labeled by each tracer were also segregated. In one case, as many as 10 distinct patches of label ranged in size from 2x2 to 5x5 mm. Each patch was segregated from the others in the region extending from the lip of the STS ventrally to the parahippocampalis gyrus. Area PITv, and possibly V3A and V4B, appeared to contain more than one patch of label. These patches were segregated by different functions.

3. The results presented here suggest that the functional division of visual pathways originates from the time when V4 is segregated from V2 and retains a different functional architecture which may reflect segregated processing of form and color information. Supported by a grant from the Whitaker Foundation and an Alfred P. Sloan Foundation Fellowship to DJS.


Visual pathways originating in cytochrome oxidase thin- and inter-stripe compartments of V2 remain segregated when termining in V4 (DeYoe et al., '92). To determine if projections from other visual areas to V4 are also segregated, different fluorescent retrograde tracers were injected at 3 sites in V4 spaced 3.3 mm apart. The tracer, briefly described above, was injected into area V4. In others, labeled zones in V4 were larger and irregularly shaped and may reflect labeling of the same module at different loci. Within occipito-temporal cortex, cells labeled by each tracer were also segregated. In one case, cats were reinforced with periodic food reward for remaining in a state of general alertness and for tolerating head restraint. No other aspect of their behavior was reinforced. Visual stimuli were presented in a visual field maintained by computer control. Enhancement of the visual response was not attributable to simple superimposition of a motor burst on the visual burst because it occurred even on trials when the saccade was delayed by several hundred msec. I conclude that the strength of the neuronal response in Area 7 is a predictor of whether a stimulus will or will not capture attention.
NEURONS WITH RELATED STIMULUS SELECTIVITY ARE CLUSTERED IN THE MACAQUE INFEROTEMPORAL CORTEX.


In neurons anterior to the 2/3 of the inferotemporal cortex (IT) of the macaque the neurons are selectively activated by visual stimuli with particular ‘complex’ features: different shape or combinations of texture or color with shape. Simultaneous recordings from 39 cells in primary visual cortex have shown that adjacent cells have similar, but usually not identical, stimulus selectivity (Fujita et al., Soc Neurosci Abstr, 1990). To assess the spatial arrangement of neurons with similar stimulus preferences we made kinds of penetrations in IT, one directed vertically and the other tangentially to the cortical surface. Recordings were made in anesthetized and immobilized monkeys (Macaca fascata).

Vertical penetrations, electrodes were advanced from the side anteriorly at an angle of 45° to the midline and downward by 27°. We presented various paper cutouts and tents of 3D objects to the monkey for the initial survey to find stimuli which activated a cell, and then determine the stimulus feature critical for the activation with the aid of a computer graphics system. We made a set of 8 to 18 stimuli (test set) including optimal, suboptimal and ineffective stimuli for that cell. Other cells were sampled at 100 or 200 μm steps in that penetration and tested with this same set of stimuli. Over a distance of 0.1-1.4 mm we obtained neurons which responded during the delay between S1 and S2 increased with the distance from the stimulus, and the number of nonmatching stimuli was different between the two recording sessions. Based on the response, a new set of stimuli was used to probe the neuron. The responses to S1 between the two recording sessions yielded no significant difference in the response strength, response variance, response latency or orientation sensitivity. However, two task-related response properties changed in the course of training. First, significantly more cells responded stronger to S1 than to the physically identical S2 in the first than in the second recording session. In the second recording session the distribution of the difference in responses to S1 and S2 was similar to that of the extensively trained monkey Ronnie. Second, the number of cells that were responsive during the delay between S1 and S2 increased with the training. These results suggest that the anterior IT is composed of modules, in which neurons with related selectivity cluster across cortical layers.

The results suggest that many IT neurons retain short-term, dynamically updated memory traces and that their responses to stimuli are modulated according to their similarity to the trace. People can also judge how well stimuli match items in a previously learned list, and this was also found to be true for the anterior IT. To examine this, two rhesus monkeys were trained to perform a “matching-to-sample” task while maintaining fixation. The sample and matching test stimuli were completely familiar to the animals, but different in shape. The responses of a third of the cells in anterior IT cortex declined steadily with increasing familiarity, reaching a plateau by about 8 presentations of a given sample. The remaining showed either no change in response or a very weak increase. The response decrement was stimulus specific. For a given stimulus, the magnitude of cumulative response decrement on a given trial was related to the recency of presentation of that stimulus in the sequence of trials. A few cells were tested with a new set of stimuli and the data showed a recovery of response to the new set. The results suggest that as stimuli become familiar, connection weights in IT cortex adjust adaptively, resulting in a narrowing of activation across the population.

PEPTIDES: PHYSIOLOGICAL EFFECTS IV

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) INCREASES CYTOSOLIC FREE CALCIUM IN CULTURED RAT HIPPOCAMPAL NEURONS. I. Yada, T. Tamura, B. Shevans and A. Adams, USA.

Japan Biomedical Research Laboratories, Tulane University Health Center, Belle Chase, LA 70037; Departments of Medicine, Physiology and Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

PACAP, a 38-residue peptide, is a new member of the vasoactive intestinal polypeptide (VIP) / glucagon / secretin family of proteins and stimulates adenylate cyclase in primary cultures of rat pituitary cells, neurons and astrocytes. PACAP, derived from a 176-amino acid precursor, is expressed in primate, sheep and rat brains. Immunoreactive PACAP nerve fibers and PACAP binding sites have been detected in the cerebellum and underline their importance in gene regulation in differentiated neurons. We demonstrate that granular neurons from rat cerebellum maintained in primary culture express functional adrenergic and CRH receptors. Activation of these receptors by Isoprenalin (5·10-6M) and CRH (10-9M) increase cAMP levels and stimulate c-fos mRNA accumulation. The role of protein kinase A (PKA) was analyzed by gene transfer studies and expression of a mutated subunit of PKA lacking cAMP binding sites. Introduction of this dominant inhibitory mutant which inactivates PKA activity results in down-regulation of PKA activity. These findings indicate that c-fos expression from chimeric genes bearing human c-fos promoter reporter constructs is increased in PKA deficient cells and that PKA activation leads to decreased transcription.

CORTICOTROPIN RELEASING HORMONE (CRH) AND CORTICOTROPIN RELEASING RECEPTORS REGULATE GENE TRANSCRIPTION IN CEREBELLAR NEURONS. J.P. Lofdahl*, F. Barthel*, A. Bouillet* and P. Fehr, Institut de Physiologie et de Chimie Biologique URA 1446 du CNRS, 21 rue René Descartes 67 084 STRASBOURG CEDEX FRANCE.

We demonstrate that granular neurons from rat cerebellum maintained in primary culture express functional adrenergic and CRH receptors. Activation of these receptors by Isoprenalin (5·10-6M) and CRH (10-9M) increase cAMP levels and stimulate c-fos mRNA accumulation. The role of protein kinase A (PKA) was analyzed by gene transfer studies and expression of a mutated subunit of PKA lacking cAMP binding sites. Introduction of this dominant inhibitory mutant which inactivates PKA activity results in down-regulation of PKA activity. These findings indicate that c-fos expression from chimeric genes bearing human c-fos promoter reporter constructs is increased in PKA deficient cells and that PKA activation leads to decreased transcription from chimeric genes bearing human c-fos promoter reporter constructs.
512.3 PHK-TYR is NECESSARY AND SUFFICIENT FOR ALPHA-BAG CELL PEPTIDE INHIBITORY ACTION ON ABDOMINAL GANGLION LUQ NEURON RESPONSES. J. BURKE, G. BURKE, D. TATMAN. Department of Physiology, University of Maryland, College Park, MD 20742.

The ganglion cell inhibited by the alpha-BAG cell is identified as 3° lateral inhibitory neurons in the ventral lateral column (VLC) of the guinea pig. The responses of these neurons to AP stimulation of the alpha-BAG cell were compared to those of neurons in the VLC that were inhibited by the A3 cell, the only other cell type that has been reported to inhibit VLC neurons.


Intracerebroventricular (ICV) NPY increases basal acid output in dogs. ICV NPY has also been shown to increase gastric acid secretion in rats with chronic cerebroventricular guides and gastric fistulas received [Leu11, Pro12]-NPY (250, 500 pmol/kg), a Y1 post-synaptic receptor agonist, or control intraluminal perfusion in rats with chronic cerebroventricular guides and gastric fistulas received [Leu11, Pro12]-NPY (250, 500 pmol/kg), a Y1 post-synaptic receptor agonist, or control intraluminal perfusion in rats. In this study, the interaction between ICV NPY and central adrenergic control of gastric acid secretion was assessed. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model. In this study, the interaction between ICV NPY and central adrenergic control of gastric acid secretion was assessed. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model. Decreases in gastric acid secretion were measured during the 30 min of perfusion, suggesting there is tonic α2-adrenergic inhibition of acid secretion in this model.
513.1 ALTERNATIVE SPlicing OF THE PARANEOPLASTIC OSPCLONUS GENE WITHIN SPECIFIC REGIONS OF THE HUMAN BRAIN. E.B. Ratcliff, Dept. of Neuro-Oncology, Memorial Sloan Kettering Cancer Center & Cornell Univ Med Ctr, NY, NY 10021

Paraneoplastic ophthalmoplegia (PO) is a neurologic disorder in which brainstem and cerebellar dysfunction are associated with malignancies. In 8 PO patients an antibody (anti-Ri) directed against their own tumors and a nuclear neuronal antigen (Ann Neurol 1989; 29:241-241) has been reported. We now have identified a cDNA encoding a protein recognized by anti-Ri antisera, and additional clones encoding what is most likely the full length of the Ri protein.

Because the neuropathic manifestations of PO are restricted, while Ri antisera recognizes an antigen expressed throughout the brain, we have characterized Ri cDNAs from different areas of the brain. A number of Ri cDNAs appear to result from splicing of novel exons into a common transcript. One such exon ("C1"), occurs within the coding region of the Ri fusion protein, is Ca2⁺ rich in serine and proline. This exon is absent from all subcortical (cerebellar and brainstem) and present in all cortical (frontal and hippocampal) cDNAs characterized. Thus the Ri gene transcript appears to be alternatively spliced in a regionally specific manner in the nervous system. If exon C1 alters the recognition of the Ri fusion protein by PO antisem, it would suggest that regional variations in the Ri antigen might contribute to the specificity of the neurologic symptoms.

513.2 MOLECULAR CLONING OF IMMORTALIZED MOTOR NEURON HYBRID CELL-SPECIFIC cDNAs. S.H. Pasternak, N.R. Pfaff, Lab of Neurobiology and Behavior, Rockefeller University, New York, NY 10021

We have pursued a strategy which could lead to the identification of motor neuron hybrid cell lines based on immortal hybrid cell lines produced by the fusion of N18TG2 neuroblastoma X dissociated spinal cord cell fusions (Soc Neurosci Abs 1989). One of these hybrid cell lines, N5C4, expresses many motor neuron characteristics not found in the parental neuroblastoma, including the ability to extend processes, to contact cultured myotubes and induce aggregation of acetylcholine receptors, and to express choline acetyltransferase, neurofilament proteins and NCAM. A CDNA library was made from N5C4, and 12 clones were isolated from it using differential and subtractive screening strategies designed to isolate CDNA copies of RNAs expressed at higher levels in the hybrid cells than in the N18 parent. Sequence analysis indicates that three transcriptional components contribute to the N5C4-enriched RNAs. These are 1. mRNAs transcribed from the mitochondrial genome, 2. small RNAs, presumably transcribed by RNA polymerase III, which correspond to the B1 highly repetitive DNA sequence element, and 3. typical cytosolic mRNAs presumably transcribed by RNA polymerase II. Among the latter group (5 clones) two have been identified as mRNAs encoding chloromarginin B (a component of neurosecretory vesicles and GAP-43, important in axonal growth cone function). The remaining three represent previously unreported sequences. We are currently investigating the tissue expression patterns of these isolated cDNAs in order to assess their expression in motor neurons.


We have used a herpes simplex virus type 1 (HSV1) defective viral vector for the transfer and expression of a foreign gene in the adult rat brain in vivo. The vector genome consists of multiple copies of an HSV1 ampiclon, a plasmid-based cloning and amplifying construct which contains HSV1 cis-acting components necessary for replication and expression. Thus the Ri gene transcript appears to be alternatively spliced in a regionally specific manner in the nervous system. If exon C1 alters the recognition of the Ri fusion protein by PO antisem, it would suggest that regional variations in the Ri antigen might contribute to the specificity of the neurologic symptoms.

513.4 EXPRESSION OF CHLORAMPHENICOL ACETYLTTRANSFERASE FROM THE HUMAN MAO B PROMOTER. R. M. Demeny and Abha Sharma. Dept. of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

Neuroamine oxidase (MAO) B oxidizes dopamine and β-phenylethylamine and activates the neurotoxin, MPTP. The X-linked, MAO B gene exhibits cell type-specific expression which differs from the similar and closely-linked MAO A gene. The MAO B promoter has not been functionally characterized. A 174 bp genomic fragment, which extends from an upstream Pst I site to a Sau 3A site in even translated region of exon 1, and which contains a TATA box-like sequence and a GC-box, was ligated in native orientation immediately 5' to a promoterless chloramphenicol acetyltransferase (CAT) gene in each of two vectors, each containing and one lacking an SAV4 enhancer. Transient expression of the enhancer-containing construct led to detectable CAT activity based on intact hybrid cell lines. The MAO B activity and mRNA. No CAT activity was detected in HeLa transfected with an enhancer-containing CAT plasmid lacking an enhancer. CAT vectors containing DNA extending further 5' from MAO B exon 1 are being tested to further define the promoter and to search for possible linked enhancer activity in the DNA region 5' to the MAO B gene. [Supported by NS 15943 and funds from the John Sealy Memorial Endowment, UTMB.]
513.5 TRANS-ACTING ELEMENTS AND OLFACTORY NEURON GENE TRANSCRIPTION. C. Stein-Izhak, M. Grillo, C. Beinhövel, M. Sakai*, L. Corner, Laboratory of Molecular Neuroendocrinology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada H3A 1A7.

Olfactory marker protein (OMP) is an abundant, phylogenetically conserved gene product of mature olfactory neurons. The OMP gene lacks introns and CAAT or TATA box motifs characteristic of genes transcribed by RNA pol II. The pattern of OMP expression argues that it may represent a class of neuron-specific genes with novel transcriptional regulation. Previously, we described a 5' segment of the OMP gene that interacts only with extracts of olfactory epithelium (OE) (C. Stein-Izhak, M. Grillo, F. L. Margolsky: Soc. for Neurosci., Abstr. 16, 1556, 1990). DNA footprinting has identified a novel 116 bp motif as the binding site of this OMP gene binding protein (OMP-GBP). OMP-GBP activity is developmentally regulated and responds to olfactory nerve lesion. OMP-GBP clusters from hepatic-arrested chronomathy at 0.4 M KCl. A putative SP1 site is located just 3' of the OMP-GBP sequence, but nuclear protein enriched extracts from rat OE lack an SP1 shift in gel mobility shift assays. These extracts do contain activities that bind to consensus sequences for Oct-1, NFκB, NF-1, and AP-1. In transgenic mice 3 Kb but not 0.3 Kb 5' upstream OMP sequences specifically activate a specific reporter gene expression. Since the 0.3 Kb upstream sequence includes the novel footprint motif additional upstream regulatory elements are being sought.

513.7 POSITIVE AND NEGATIVE REGULATORY CIS-ACTING ELEMENTS IN GLUTAMINE SYNTHETASE EXPRESSION. B.J. Patschke*, R.G. Kluver*, D.E. McCullough, V.P. Reiter*, and S.E. O'Connor*, Lab. of Molecular Biology, MINDS, NIR, Bethesda, MD 20892.

Glutamine synthetase (GS) gene expression is confined to astrocytes in the CNS. The gene for GS was cloned and the 5' flanking region isolated. A series of deletion and site-directed mutants of the promoter region were generated by PCR, cloned into psiCAT, and expressed in various cell lines. We identified three regions of importance for the regulation of the GS promoter. Mutation of an enhancer expression, an SP1 site located at -222, an enhancer region at -315, and a silencer region at -797. The deletion mutants show that the AP2 and enhancer sites are sufficient for cell type specific expression. The inclusion of only the AP2 site creates a small increase in expression while a large increase was seen when the enhancer region was included. This elevation was suppressed by the inclusion of the silencer region.

Site-directed mutagenesis studies demonstrate that while the AP2 site has only a small effect by itself, the enhancer region requires the AP2 site for its induction. Mutation of the silencer region leads to an increase in expression, though dual mutation of the AP2 and silencer sites further increase the promoter activity, suggesting that all three sites are interacting in controlling GS expression.

513.9 IDENTIFICATION OF A RETINOIC ACID RESPONSE ELEMENT IN THE HUMAN OXYTOCIN GENE PROMOTER WHICH OVERLAPS WITH AN ESTROGEN RESPONSE ELEMENT. Stéphane Richard* and Hans H. Zingg, Laboratory of Molecular Endocrinology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada H3A 1AT.

We previously identified an estrogen (E2) response element (ERE) at position -145 bp from the human oxytocin gene promoter (JBC 265:6088). By transient expression of OT 5'-flanking sequences linked to the CAT gene, we now show that retinoic acid (RA) elicits a 7-fold stimulation of the transcriptional activity of the OT promoter in neuroblastoma cells co-transfected with either the human RA receptor (RAR) α, β, or γ. We further demonstrate that the presence of 4 TGGAC repeats, located at -162, -156, -103 and -83, is an absolute requirement for maximal RA induction. Since the TGGAC repeats at -162 and -156 are contained within the ERE (GGTCACTGACC), we next determined the level of synergy and/or interference that occurs when both hormones are added together. In constructs containing an intact ERE and all 4 TGGAC repeats, RA and E2 had an additive effect. By contrast, in constructs in which the TGGAC repeats at -103 and -83 were deleted, RA decreased E2-induced stimulation by ~50%. A likely interpretation of these data is that, in the absence of the two downstream TGGAC repeats, the RAR is forming a transcriptionally inactive complex with the ERE and is thereby preventing the ER from binding to the ERE. The overlapping elements in the human OT promoter create a natural molecular model system for the investigation of interactions between nuclear factors.

513.6 FUNCTIONAL REGULATION OF PROGESTERONE RECEPTOR TRANSCRIPTIONAL ACTIVITY BY PHOSPHORYLATION. L. Denner, N. L. Wester*, O. M. Connolly* and B. W. O'Malley*, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Phosphorylation regulates transcriptional activity of the progesterone receptor (PR) by ligand-dependent and ligand-independent mechanisms. We have used single amino acid substitution mutagenesis of serine S530 (S530), the progesterone-induced phosphorylation site, to elucidate the precise role of phosphorylation in functional regulation of PR. S530 was mutated to glycine (S530G) to prevent phosphorylation and to aspartic acid (S530D) to constitutively provide negative charge, thus partially mimicking the changes induced by phosphorylation. All receptor forms had equal hormone binding and expression levels in transient transfections of CV1 cells. S530 and S530G had similar maximal transcriptional activation of the PRE-CAT reporter gene while S530D was reduced 25-50%. Gel retardation studies using the PRE showed that S530D had substantially higher DNA binding than S530 and S530G, which were similar to each other, indirect immunofluorescence indicated that S530 and S530D were localized exclusively to the nucleus after treatment with either progesterone or okadaic acid, a phosphatase inhibitor that activates PR-mediated transcription in the absence of progesterone by the ligand-independent pathway. S530D was perinuclear under all conditions. These observations suggest that the ligand-dependent pathway for transcriptional activation of the PR occurs by phosphorylation of S530, resulting in an increase in DNA binding activity. The ligand-independent pathway occurs by phosphorylation of a site distinct from S530.
513.11 REGULATION OF TISSUE- AND TREATMENT-SPECIFIC ppENK RNA INITIATION. G. Welsinger, J.D. DeCristofaro and Edmund F. La Gamma. Department of Pediatrics and Neurobiology, SUNY at Stony Brook, Stony Brook, NY 11794.

We previously demonstrated that rat preproenkephalin (ppENK) RNA is initiated from at least 4 start sites by primer extension and S1 analysis of total RNA (MRC 265:17389, 1990). To further compare specificity of usage, we surveyed 12 tissues in the basal state using primer extension analysis. E3 + E4 sites accounted for 80% of the 1.4 kb striatal RNA species compared to a preferential usage of the E2 site in all other tissues; including the brain. The E1 site was selectively absent in striatal RNA. To evaluate effects of induction on usage, rats were given cholinergic drug treatments (nicotinic 5 mg/kg + metoxamine 1 mg/kg; 2q 12h up to 4d). Adrenomedullary E2 transcripts peaked at 2d increasing 5-fold while newly induced E4 transcripts increased 80 fold. In contrast, after hypoglycemia (insulin 10/kg sc, rescue at 2h with 40% sucrose PO), only the E4 transcript was increased (16-fold). Interestingly, in both treatment paradigms, steady state levels of adrenal ppENK mRNA increased 50 to 100 fold. In the striatum of the cholinergic treated groups, RNA initiation increased 10-15 fold but not after insulin treatment (hypoglycemia). These studies demonstrate tissue and treatment specific differences in ppENK RNA start site usage and steady state levels of mRNAs. (NHF 89NS0719872.)


A 13.4 kilobase genomic fragment of the bovine vasopressin (VP) gene was micro-injected into mouse oocytes. Your independent transgenic mouse lines were derived and three lines had been assayed for tissue distribution of bovine VP RNA. All lines showed bovine specific expression in testes. In addition, two lines showed bovine VP RNA in the hypothalamus. In situ hybridisation of transgenic mouse hypothalamic sections identified bovine VP RNA expressing cells in neurons of the supraoptic nuclei. When the transgenic animals were subjected to an osmotic stimulus (29% NaCl in their drinking water for 7 days), the bovine message level increased 1.6 fold, similar to that of the endogenous murine message. These studies show that the 13.4 kilobase bovine genomic vasopressin fragment contains at least some of the regulatory elements necessary to direct tissue-specific expression and physiological regulation in the transgenic mouse hypothalamus.

FORMATION AND SPECIFICITY OF SYNAPSES V


The synapsins are a family of neuronal phosphoproteins associated with synaptic vesicles and believed to be involved in the regulation of neurotransmitter secretion. In the developing nervous system, the expression of synapsin I correlates with synaptogenesis and synapsin I and IIb cDNA results in marked increases in the number of nerve terminals per cell, in the number of synaptic vesicles per terminal, and in the number of synapse-like contacts. We have now found that synapsin I, loaded into Xenopus embryos, promotes the functional maturation of developing neuromuscular synapses in culture. Spontaneous synaptic currents occurred with higher frequency and their amplitudes showed an earlier appearance of bell-shaped distribution indicative of more mature quantal secretion. Impulse-evoked synaptic currents also showed a significant increase in amplitude. These results provide physiological evidence for the involvement of synapsin I in the maturation of neuromuscular synapses.

514.3 MOTOR NERVE TERMINALS ARE SELECTIVELY ELIMINATED FROM REGIONS OF NEUROMUSCULAR JUNCTIONS FOCALLY BLOCKED WITH α-BUNGAROTOXIN. R.J. Balice-Gordon and J.W. Lichtman. Dept. of Biology, Wesleyan Univ., Middletown, CT 06457.

Following innervation neuromuscular junctions over time in living mice has shown that the postsynaptic ACh receptor regions beneath one motor axon are selectively maintained while those beneath the other motor axon lose motoneuronal terminals (Charlton, 1990), however, the mechanisms that give rise to this channel distribution are unknown. Using cultured Helisoma involvulus neurons, our goal is to determine the regulatory mechanisms controlling calcium channel distribution during synaptogenesis.

Individual neurons were plated into culture, and, after 1-3 days, action potential- evoked calcium transients were imaged using Fura-2. Trains of action potentials reliably evoked homogeneous calcium transients throughout the neurite. Single, dissociated muscle fibers from the supralateral radial lobe (SLT) muscle, the normal effector muscle of neuron B19, were plated onto neurites and allowed to contact overnight. Action potentials in sympathetically connected neuromuscular preparations evoked a heterogeneous pattern of calcium transients, with the largest calcium transient being localized to the site of muscle contact. We have begun to address the mechanism(s) underlying the focal calcium transients by first determining the time course of the transition to the heterogeneous state. Neurons were plated alone, in culture, and the calcium transients shown to be homogeneously distributed. Single muscle fibers were then plated on to the neurites, and by imaging sequential calcium transients, a transition to focal heterogeneity was determined to occur within 30 minutes of muscle contact.
SUPPRESSION OF DEVELOPING NEUROMUSCULAR SYNAPSE BY FOCAL IONTOPHORETIC APPLICATION OF ACETYLCHOLINE. Y. Dan and M. Poe, Dept. of Biological Sciences, Columbia Univ., N.Y., N.Y. 10021

Brief tetrani stimulation of a presynaptic neuron leads to rapid functional suppression of synapses made by other co-innervating neurons on the same postsynaptic muscle cell (see preceding abstract). In the present study, we tested the hypothesis that such heterosynaptic suppression was due to repetitive postsynaptic activation of myocyte acetylcholine (ACH) receptors by the stimulated neuron. We have examined the effect of repetitive focal iontophoretic application of ACH on the synaptic efficacy at a single-innervated myocyte in 1-d old Xenopus cultures, using whole-cell voltage-clamp recording. The amplitude, duration and frequency of iontophoretic currents were adjusted to produce ACh-induced membrane currents of similar characteristics as those generated by ACh release from a tetanized neuron. We found that application of 100 pulses of ACh (at 2 Hz), each induced an inward current of peak amplitude of 1 to 3 nA and a duration of about 50-70 ms, consistently led to suppression of the synapse if the site of the ACh application was within 10-15 μm from the synapse. The suppression was not due to the desensitization of the ACh receptors by the ACh release during tetrodephoresis. Both the proximity requirement as well as the persistence of suppression following the repetitive stimulation are similar to the heterosynaptic suppression induced by nerve stimulation. The cellular events underlying the ACh-induced synaptic suppression, the involvement of Ca influx through the activated ACh channels in particular, remain to be investigated.


Inteurneurons MGI and 10-3 of the Cricket terminal abdominal ganglion have different response properties. MGI responds phasically to a 100 Hz sound stimulus while 10-3 responds tonically. This phasic/tonic difference is believed to reside in the properties of the sensory to interneuron synapses since neither accommodation of the sensory receptor nor adaptation of the sensory neuron can account for the phasic response of MGI. EPSPs recorded in the dendrite of MGI initially facilitate dramatically and then show pronounced response decrement during high frequency, 100-150 Hz, stimulation of single interneurons. We believe this high frequency nervous plasticity is the basis for the plastic response of MGI and that synapses on 10-3 lack this plasticity.

To determine how synapses with different release properties are specified from a single population of sensory neurons on two different interneurons we have examined an identified sensory neuron (SN 3c) which simultaneously contacts both MGI and 10-3. 100Hz stimulation of SN 3c evokes EPSPs in both MGI and 10-3 which facilitate dramatically followed by pronounced response decrement while EPSPs simultaneously evoked in 10-3 are more stable, neither facilitating nor decrementing dramatically. We hypothesize that the post-synaptic interneurons have a role in determining the release properties of their preterminal synapses. We are currently attempting a quantal analysis of these synapses and investigating the role of post synaptic activity in determining pre-synaptic release properties. Supported by NSF grant # BNS 90-96180.


The glutamate receptor of the NMDA type has been reported to be involved in axonal remodelling in the immature visual system. During the postnatal development of the rodent cerebellum, the granule cells and the Purkinje cells exhibit a transient sensitivity to NMDA receptor antagonists. Therefore, the cerebellum offers a unique model to test the hypothesis that the NMDA receptor participates in synapse elimination. In the present study, a selective antagonist of this receptor, the D,L-APV, was infused tonically from a slice of Elvax polymer implanted on rat cerebellar surface during the whole period of synapse elimination (PN4-PN17). The degree of innervation of Purkinje cells by climbing fibers was then analysed by in vivo intracellular recording.

On 183 Purkinje cells recorded from 31 rats treated with D,L-APV, 47.5% remained innervated by 2 or 3 climbing fibers. By contrast, only 17.3% of 75 Purkinje cells recorded in 10 rats treated with the inactive isomer L-APV remained similarly polynerviated; the polyinnervation persisted also in only 11.1% of the 57 Purkinje cells recorded from 8 rats implanted with Elvax alone.

These data suggest that the NMDA receptor plays an important role in the synapse elimination process, probably related to the modulation of Purkinje cell electrical activity by its afferents.


The hallmark of Parkinson's disease is a major loss of dopaminergic neurons, causing a marked reduction in the concentration of dopamine in the corpus striatum. The surviving neurons are able to compensate by enhancing the rate of dopamine synthesis. Furthermore, it has been postulated that this may result in accumulation of free radicals within the terminals, which may be toxic or lethal to the neurons. Indeed, studies by various groups have shown that dopamine neurons have increased susceptibility to free radicals. We have recently shown that dopamine neurons possess an increased susceptibility to free radicals, which is increased by the removal of iron and transferrin in the substantia nigra of Parkinson's patients, along with the beneficial effects of α-tocopherol and/or L-deprenyl as additional suggestive evidence. Our laboratory has discovered and characterized metallothionein (MT) in several regions of mammalian brain, including striatum. Zinc, which is able to remove superoxide and displace iron from the redox catalyst on metallothionein, is selectively reduced in 6-hydroxydopamine (μg in 4 μl) of 0.02% ascorbic acid-lesioned rat striatum. Similarly, zinc, with its metallophilic sulfhydryl group able to interact with iron, may be a key factor in regulating the intracellular redox potential, which is depleted in conditions causing destruction of striatal neurons. Moreover, neuroblastoma IMR-32 cells, unable to synthesize large amounts of MT in culture, exhibit far greater vulnerability to metal toxicity when compared to Chang liver cells, which synthesize copious amount of MT. The results of these studies are critical to indicate that zinc and MT may be involved in oxidative stress in Parkinson's disease. (Supported in part by a grant from USPHS ES-0349).
THURSDAY PM
PARKINSON'S DISEASE

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515.3

Neurons expressing 28kd Calcium Binding protein (CaBP) and tyrosine hydroxylase (TH) were analyzed immunohistochemically in midbrains from 3 controls and 3 patients with Parkinson's disease (PD). Double labeling experiments showed that all TH-positive neurons were CaBP-positive in the dorsal mesencephalon (ventral tegmental area, substantia nigra and ventral midbrain region A8) and that only 10 to 20% of the CaBP-positive neurons were TH-negative. In the ventral mesencephalon (substantia nigra and ventral tegmental area) only rare CaBP-positive TH-positive neurons were detected, whereas most TH-positive neurons were CaBP-negative.

In PD, the loss of TH-positive neurons was moderate in the dorsal part and severe in the ventral part of the mesencephalon. The number of CaBP-positive neurons was not different in the midbrain of controls and parkinsonian patients. The results suggest that CaBP may contribute to the protection of catecholaminergic neurons that are not affected by pathological process in PD. Supported by INSERM and NIH (# NIH NS 25229).

515.4
Early stages in nerve cell degeneration in MPTP-induced Parkinsonism in the squirrel monkey. L.S. Forno, L.E. Delaney, J. Irwin, M. Branski, and J. B. Papp. The VA Medical Center, Palo Alto, CA 94304, and the California Institute for Medical Research, San Jose, CA 95128.

Three 10-12 year-old squirrel monkeys were anesthetized and perfused intracardially with paraformaldehyde-glutaraldehyde and examined by electron microscopy (EM) 2, 3 and 4 days after a single s.c. injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 2.5 mg/kg. In all three animals the substantia nigra (SN) displayed distended dendrites and postsynaptic structures containing membranous structures. Mitochondria were generally better preserved than other organelles. Axonal swellings in myelinated fibers were observed in 2 of 3 animals. Some nerve cells appeared normal; others showed loss of or disorganization of Nissl substance. In the striatum focal vacuolization was noted, and some nerve cell processes contained membranous material. Immunocytochemistry with antibody to tyrosine hydroxylase (TH) revealed striking preservation of TH positive fibers in the striatum in the 3 day animal, with some decrease in the 4 day animal. In the SN nerve cell loss was not observed, but proximal portions of the nigrostriatal pathway showed swollen axons in the TH-reacted tissue. The findings correlate well with the biochemical changes recently reported in squirrel monkeys that received a similar regimen of MPTP (Brain Res 1980;324:242-252).

515.5
Comparison of neurotoxic actions of MPP+ and NH2-MPP+ on the survival of peripheral adrenergic neurons of the chick embryo. J.V. Blauze, J. Johannessen*, and A.R. Wikade, Dept. of Pharmacology, School of Medicine, WSU, Detroit, MI 48201 and # FDA, Washington, D.C.

Central dopaminergic neurons are the potential targets for MPP+ and analogues. The neurotoxic effect depends on the uptake of MPP+ through the dopamine carrier system. Since sympathetic neurons (SN) demonstrate the high affinity and desipramine (DMI) sensitive norepinephrine uptake we examined the effects of MPP+ and NH2-MPP+ on their survival in culture. When MPP+ or NH2-MPP+ was added to the medium with NGF almost 15-30% of the neurons died after 2 days in culture. Toxic effect of MPP+ was evident at 3 μM and was maximum at 1 μM. ED50 for MPP+ and NH2-MPP+ was about 20 μM and 200 μM, respectively. Pretreatment of SN with DMI prevented the toxic effects of MPP+ and NH2-MPP+. Neurotoxic effect of MPP+ was not evident when it was added to the medium 24 hours after the neurons were plated. NH2-MPP+ was toxic even when added 24 or 48 hours after the start of culture. Sensory neurons were unaffected by as high as 10 μM MPP+ or NH2-MPP+. Our studies show that primary cultures of chick SN could serve as an effective model to explore the mechanism of neurotoxic action of MPP+ and related compounds.

515.6
Chronic Nicotine Enhances the Dopaminergic Neurotoxicity of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine in Black Mice. R.A. Behrends and G.L. Harle. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

There is a low prevalence of Parkinson disease among cigarette smokers. Nicotine is a major component of cigarette smoke. Thus, we examined the effect of chronic nicotine on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity. Adult male C57 black mice (25 g) were implanted subcutaneously with Alzet minilogs loaded with 15-20 mg nicotine that discharged their loads over 14 days. Control mice were not given nicotine. Seven days later, MPTP was injected subcutaneously, and their striatal tissue was analyzed for dopamine and its metabolites by high-performance liquid chromatography with electrochemical detection. We found that nicotine at all doses significantly enhanced MPTP-induced reduction in striatal dopamine and its metabolites. Also, at the higher MPTP doses, several of the nicotine-acclimated mice died while none of the control mice died. The results strongly suggest that nicotine accentuates rather than reduces MPTP neurotoxicity.

515.7
Changes in Brain Catecholamines and Dopamine Uptake Sites at Different Stages of MPTP Parkinsonism in Monkeys. G.M. Alexander, R.J. Schwartzman, S. Irwin, S. Bolt, L. DeLanney, and J.W. Langston. VA Medical Center, Palo Alto, CA 94304, and the California Institute for Medical Research, San Jose, CA 95128.

MPTP is classified as an autosomal recessive mutation because its behavioral phenotype is visible only in homozygous weaver mice. However, many cellular and biochemical effects of weaver are less severe in the homozygous weaver than other organanelles. Axonal swellings in myelinated fibers were observed in the 2 day animal. Some nerve cells appeared normal; others showed loss of or disorganization of Nissl substance. In the striatum focal vacuolization was noted, and some nerve cell processes contained membranous material. Immunocytochemistry with antibody to tyrosine hydroxylase (TH) revealed striking preservation of TH positive fibers in the striatum in the 3 day animal, with some decrease in the 4 day animal. In the SN nerve cell loss was not observed, but proximal portions of the nigrostriatal pathway showed swollen axons in the TH-reacted tissue. The findings correlate well with the biochemical changes recently reported in squirrel monkeys that received a similar regimen of MPTP (Brain Res 1980;324:242-252).

515.8

Weaver is classified as an autosomal recessive mutation because its behavioral phenotype is visible only in homozygous weaver mice. However, many cellular and biochemical effects of weaver are less severe in the homozygous weaver than in homozygous normal mice. Methyl dopaminergic uptake in the homozygous weaver confirmed and extended previous findings of dopamine uptake in the homozygous weaver. In the dorsal and ventral caudoputamen of adult homozygous normal, homozygous weaver and homozygous weaver mice, the effect of MPTP on dopamine uptake was measured in synaptic preparations of the dorsal and ventral caudoputamen of adult homozygous normal, homozygous weaver and homozygous weaver mice. The effect of MPTP on dopamine uptake in the homozygous weaver confirmed and extended previous findings of dopamine uptake in the homozygous weaver. We suggest that these defects may lead to cell death in the homozygote where they are present in two doses. NS20181; MH40655 and the Parkinson’s Disease Foundation.

515.9
Changes in Brain Catecholamines and Dopamine Uptake Sites at Different Stages of MPTP Parkinsonism in Monkeys. G.M. Alexander, R.J. Schwartzman, S. Irwin, S. Bolt, L. DeLanney, and J.W. Langston. VA Medical Center, Palo Alto, CA 94304, and the California Institute for Medical Research, San Jose, CA 95128.

MPTP is classified as an autosomal recessive mutation because its behavioral phenotype is visible only in homozygous weaver mice. However, many cellular and biochemical effects of weaver are less severe in the homozygous weaver than other organanelles. Axonal swellings in myelinated fibers were observed in the 2 day animal. Some nerve cells appeared normal; others showed loss of or disorganization of Nissl substance. In the striatum focal vacuolization was noted, and some nerve cell processes contained membranous material. Immunocytochemistry with antibody to tyrosine hydroxylase (TH) revealed striking preservation of TH positive fibers in the striatum in the 3 day animal, with some decrease in the 4 day animal. In the SN nerve cell loss was not observed, but proximal portions of the nigrostriatal pathway showed swollen axons in the TH-reacted tissue. The findings correlate well with the biochemical changes recently reported in squirrel monkeys that received a similar regimen of MPTP (Brain Res 1980;324:242-252).
515.9

ROTATIONAL BEHAVIOR ALTERATIONS FOLLOWING INFUSION OF GABAERGIC AGENTS INTO THE SUBTALAMIC NUCLEUS OF RATS WITH UNILATERAL NIGRAL LESIONS


GABAergic fibers from the basal ganglia regulate activity in the STN which in turn regulates the internal segment of the globus pallidum (GPI) via glutamatergic fibers. Lesions of the STN have been shown to reduce MPTP-induced parkinsonism in primates. We infused GABAergic agents into the STN and monitored apomorphine-induced contralateral rotation (APO-CTR) in five, 6-hydroxydopamine (6-OHDA) lesioned rats to test whether modulating output affects rotation in this model. Using 0.5 μl/2.5 min injections through implanted guide cannulae the STN was infused with normal saline (NS), the GABA-A agonist muscimol (200 ng), or the GABA-A antagonist bicuculline (200 ng) at 2 day intervals. Bicuculline decreased APO-CTR 79%, NS did not alter APO-CTR, while muscimol increased it 157%. It appears that GABA outflow neurons from the striatum regulate activity in the STN which, in turn, influences the outflow from the GPI. Reducing the activity of the STN in the parkinsonian brain using drugs might therefore be beneficial in the treatment of Parkinson's disease.

515.10


Dementia in idiopathic, Lewy body positive, Parkinson's disease (PD) has been reported to be correlated with neuronal loss in the nucleus basalis (NB), substantia nigra pars compacta (SN) (medial part), and locus coeruleus (LC), and with the presence of Lewy body-like (LB) inclusions in neocortex. We counted neurons at selected anatomical levels of the LC and SN, and rated NB neuronal loss and LB numbers in the anterior cingulate (ubiquitin immunocytochemistry), in 13 patients with pathologically confirmed PD without concurrent Alzheimer's disease. Individuals with dementia (7 patients, mean age 76.0) had significantly fewer neurons at all levels of the LC, but not the SN, greater NB neuronal loss, and more anterior cingulate LBs than those without dementia (6 patients, mean age 71.5). In conclusion, dementia in PD reflects patterns of extra-nigral neuronal involvement.

515.11

CELLULAR EXPRESSION OF CATECHOL-O-METHYLTRANSFERASE (COMT) IN RAT CNS AND PERIPHERAL ORGANS REVEALED BY IN SITU HYBRIDIZATION-HISTOCHEMISTRY

J.G. Richards, B. Bertocci*, F.Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland

COMT (EC 2.1.1.6), a membrane-associated enzyme, catalyzes the metabolism of dopamine, noradrenaline, adrenaline and other catechol derivatives. In the brain, COMT is responsible for the extraneuronal inactivation of catecholamines released into the extraneuronal space. CDNA clones for human and rat COMT have been recently isolated and sequenced (Bertocci et al., Proc. Natl. Acad. Sci USA 1990; 87:2478-2482). In order to identify the cells expressing COMT in rat CNS and peripheral organs, we used 35S-labelled oligonucleotide probes (nucleotide sequences 92-152 and 587-647) for in situ hybridization histochemistry. Transcripts were found in discrete regions of rat brain (ependyma, choroid plexus, circumventricular organs, dentate gyrus granule cells > hippocampal CA1-4 pyramidal cells, cerebellar granule cells, Bergmann glia, brain stem nuclei - facial n., vestibular n., olivary n., pontine n. > cerebral cortex, olf. bulb), leptomeninges, extraneuronal space. cDNA clones for human and rat COMT have been recently isolated and sequenced (Bertocci et al., Proc. Natl. Acad. Sci USA 1990; 87:2478-2482).

515.12

NEOSTRIATAL DOPAMINE DERENATION FOLLOWING INTRASTRIATAL 6-HYDOXYDOPAMINE RESEMBLES WEAVER MOUSE STRIATUM.


Injury to dopamine (DA) neurons was characterized following delivery of 6-hydroxydopamine (6-OHDA) to the rat striatum. Two wks after injecting 25, or 100 μg of 6-OHDA, the density of striatal DA terminals, defined by [3H]mazindol autoradiography, was lowered by 22, 48, and 52% respectively. Losses predominated in dorsal striatum. Central-rostal (1.4-fold) and medial-lateral (1.7-fold) gradients of increasing [3H]mazindol binding in the intact striatum were abolished by 8 or 25 μg of 6-OHDA. Striatal contents of DA, 3-MT, DOPAC, and HVA and [3H]mazindol binding were each decreased by 57-86% at 1 and 4 wks after 25 μg 6-OHDA, with a partial recovery in all measures by 4 weeks. Little or no alteration in 5-HT, NE, and related compounds were observed from 1-4 weeks post-injury. 6-OHDA or vehicle increased the density of the glial marker [3H]HPK 11195 but only adjacent to the cannula tract. Immunocytochemical staining at 2 wks after 25 μg 6-OHDA showed a 31-50% decrease in the number of tyrosine hydroxylase-positive neurons at 3 levels of the central and medial pars compacta of the substantia nigra but not in the VTA. Intrastriatal 6-OHDA treatment produces a reproducible, DA neuron-selective, and anatomically limited depletion of DA nerve terminals and cell bodies that closely resembles these patterns in weaver mouse striatum. The sparing of DA cell bodies relative to terminals provides a model for neurotrophic factor rescue or sprouting of DA neurons.

515.13

PERSISTENT CONSEQUENCE OF LIMITED TREATMENT WITH L-DOPA IN RATS WITH UNILATERAL NIGRAL LESIONS.

P.B. Silberman. Dept. of Psychiatry and Behavioral Sciences, Univ. of Texas Health Science Center, Houston, TX 77030.

Rats with a unilateral 6-hydroxydopamine lesion of substantia nigra not only rotate (circle) contralaterally immediately after administration of the direct acting dopamine agonist, apomorphine, but also weeks later when they are reintroduced to the environment in which apomorphine had been administered. Here, the ability of limited L-DOPA treatment to result in similar conditioned rotation was tested. Lesioned, but otherwise drug-naive rats were administered 5 mg/kg carbipoda (ip) 30 min prior to 10 to 50 mg/kg L-DOPA (ip) and placed in hemispherical plastic bowls. Rotations in each direction were counted via a video camera and image analyzer. Two weeks after a single drug exposure, most rats briefly made rapid contralateral rotations when presented with the drug-free ipsilateral rotation bowl. As previously found with apomorphine, lower doses of L-DOPA were more effective than higher doses in conditioning this behavior in a single trial. Rats administered carbipoda + L-DOPA on three consecutive days generally showed an increased response to successive administrations (sensitization). Some thrice-treated animals also exhibited explosively rapid rotation when returned, undrugged, to the environment associated with L-DOPA. This striking rotation developed progressively over several months after the last drug treatment and may prove to be a useful model for study of abnormally involuntary movements associated with parkinsonism. (Supported by NIDA grant DA06269).
516.1

**NMDA RECEPTOR-MEDIATED MOBILIZATION OF INTRACELLULAR Ca²⁺ STORES IN PRIMARY CENTRAL NEURONS**

Zheng Z. Lei, Dongxian Zhang, and M.D. Lodge. Department of Neuroscience, Harvard Medical School, Boston, MA 02115.

NMDA receptor-mediated increases in Ca²⁺ (12 μM; APV 200 μM), magnesium (1 mM) or EGTA (2 mM) completely blocked NMDA-evoked increases in [Ca²⁺]. Also, a 2 min preincubation with 30-100 μM dantrolene, a drug that blocks Ca²⁺ release from sarcoplasmic reticulum and possibly from neuronal intracellular stores (Rios et al., Soc. Neurosci. Abstr. 1989;15:17), produced a dose-dependent inhibition of the NMDA-evoked [Ca²⁺] increase; maximal block was 60% of the NMDA response. In whole cell patch-clamp recordings, dantrolene did not inhibit NMDA-activated currents. This result is in agreement with receptor binding studies showing no direct interaction of dantrolene with glutamate receptors (Frandsen & Schousboe, J. Neurochem. 1991;56:1075). With 2 mM EGTA and no added calcium in the bath, 2 μM ionomycin produced a transient increase in [Ca²⁺], apparently depleting intracellular stores. This effect of ionomycin could be prevented by preincubation with dantrolene, consistent with an intracellular mode of action of dantrolene. Taken together, these results suggest that NMDA-stimulated Ca²⁺ influx through ion channels leads to the mobilization of Ca²⁺ stores. Hence, both extracellular and intracellular calcium pools may be important for NMDA receptor-mediated physiologic and neurotrophic events.

516.3

**ACTIVATION OF THE METABOTROPIC EXCITATORY AMINO ACID (EAA) RECEPTORS BY INTRASTRIAL INJECTION OF 1S,3R-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID (1S,3H-ACPD) INDUCES CONTRALATERAL TURNING. A. Seccom, J.A. Mohn and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285.**

1S,3R-ACPD is a selective agonist at the metabotropic EAA receptor (see Schoepp et al., this issue). In this study we have investigated the consequence(s) of activating the metabotropic EAA receptor in vivo. Unilateral intrastriatal injection of 1S,3R-ACPD produced dose-related turning behavior with a concentration-response relationship (EC50 value = 0.59 μM; 2 μl, 3 ± 0.4 rotations/5min at 1 μM; 2 μl). This effect of 1S,3R-ACPD was not mimicked by saline vehicle (2 μl) or by the less active EAA isomer 1R,3S-ACPD (2 μl). Administration of the NMDA receptor antagonist LY274614 (2.5 mg/kg i.p.) did not significantly alter the 1S,3R-ACPD (1 μM)-induced rotation. However, coinjection of the metabotropic receptor antagonist L-AP3 (1 μM) significantly reduced rotations by 1S,3R-ACPD (1 μM). Maximal effective behavioral doses of 1S,3R-ACPD in the striatum of adult rats, as indexed by glutamic acid decarboxylase (GAD) activity in the injected striatum. NMDA (0.2 μM) induced a 7 ± 6% loss of GAD activity. This suggests that 1S,3R-ACPD-induced rotations are a behavioral consequence of activating striatal metabotropic EAA receptors. Furthermore, unlike ionotropic EAA receptor activation, direct in vivo metabotropic EAA receptor activation is not associated with convulsions and/or excitotoxicity.

516.5

**NEW NON-NMDA ANTAGONIST DISCRIMINATES BETWEEN AMPA AND KAINATE RESPONSES ON CORTICAL NEURONES IN VITRO BUT NOT ON SPINAL NEURONES IN VIVO. N.G. Jones, P.L. Thomas, J. Pywell and D. Lodge. Department of Veterinary Basic Sciences, Royal Veterinary College, NW1 0TU, UK.**

We have used both application to cortical wedges and electrophoretic application to spinal wedge in halothane-anesthetised rats, to characterise non-NMDA receptors in the mammalian central nervous system with a novel benzodiazepine compound, GYK (Tarnawa et al., 1985). With cortical slices, GYK had an IC50 of 150 μM of AMPA (40 μM) of 12 ± 1.5μM, whereas responses to KA (50 μM) and NMDA (40 μM) were reduced by 50 and 20% respectively. The AMPA dose-response curve was shifted in a more or less parallel manner. On spinal neurons in vivo electrophoretic application of GYK conversely reduced responses to AMPA, KAIN and NMDA by 56 ± 27, 43 ± 28 and 17 ± 18% respectively. Similar benzodiazepine analogs may reverse the use of non-NMDA antagonists with pharmacological and therapeutic beneﬁts.


516.6

**EVIDENCE THAT NMDA RECEPTORS STIMULATE DEPHOSPHORYLATION OF DARPP-32 BY CALCINEURIN IN STRIATAL NEURONS**

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DARPP-32 is enriched in the medium-sized spiny neurons of mammalian neostriatum. In most cases, dopamine and glutamate have opposite effects on the physiological activity of these cells. One mechanism for antagonism of these interactions of glutamate and dopamine on these cells may involve DARPP-32. The phosphatase state of DARPP-32 on a pharmacologically active site (Thr-34) is increased by dopamine via activation of a calcium-dependent protein kinase. We recently showed that glutamate, via NMDA receptors, is able to antagonize this effect. We suggested that the calcium/calcmodulin-dependent protein phosphatase (PP2B, also called calcineurin) might mediate this effect. NMDA receptor activation is known to trigger an increase in intracellular calcium. Both calcineurin and PP2A can dephosphorylate Thr-34 of DARPP-32 in vitro. Okadaic acid is a compound which selectively inhibits PP2A, but not PP2B. We have treated slices of rat brain striatum with okadaic acid in order to examine whether the NMDA effect is mediated by PP2A or by PP2B. Okadaic acid increased the phosphatase state of DARPP-32 in a dose-dependent and time-dependent manner, suggesting that an okadaic acid-sensitive phosphatase does regulate DARPP-32 phosphorylation in vivo. However, okadaic acid did not inhibit the ability of NMDA to induce dephosphorylation of Thr-34. These results suggest that PP2B, but not PP2A, mediates the action of NMDA receptors on DARPP-32 dephosphorylation, and support our hypothesis that NMDA receptors are coupled, via calcium, to the activation of calcineurin.

516.8

**MULTIPLE QUISSALUP: UPTAKE SITES IN RAT HIPPOCAMPAль SITES**

Ian C. Kilpatrick* and Peter S. Harrison* (SPON: Brain Research Association).

Department of Pharmacology, University of Bristol, Bristol, BS8 1TD, UK.

Potentiation of the depolarising action of the excitatory amino acid (EAA) agonist quisqualate (QUIS), by uptake blockers the compound may be subject to uptake mechanisms. However, we have already shown an absence of active uptake of 16mM QUIS into rat hippocampal slices (Kilpatrick & Harrison (1991) J. Physiol. Lond., 435, 43P). The aim of this study was to directly investigate the accumulation of higher QUIS concentrations. In addition, since active EAA transport appears to occur via two distinct sites: a high-affinity, Na⁺-dependent carrier and a lower-affinity, Cl⁻-dependent/Ca²⁺-potentiated site, the relative effects of replacement of Na⁺ and Cl⁻ with impermeant ions has been examined. Following incubation of 400 μM QUIS with 1mM Ca²⁺ and 4mM for 5 min at 25°C, homologous levels of QUIS as measured by HPLC were 502 ± 53 ± 2871 ± 180 pmol/mg (mean ± S.E.M.; n = 7 and 8, respectively). Both values were reduced to at least 60% by complete substitution of Ca²⁺ with 2mM EDTA. This was due to a slight decrease in the apparent distribution of the Na⁺ and Cl⁻-dependent sites. However, complete replacement of Na⁺ and Cl⁻ with either ethanoate (CH₃COO⁻) or methanesulphonate (CH₃SO₄⁻) did not influence QUIS transport at this concentration (+0.4%, n = 7 and +2.1%, n = 3, respectively). At 2mM CH₃SO₄⁻, however, Ca²⁺ substitution with CH₃COO⁻ but not with CH₃SO₄⁻ (±7.5%, n = 3), induced a significant reduction in uptake of -22.7% (n = 3, P < 0.05), whereas replacement of Na⁺ offset uptake by -72.2% (n = 3, P < 0.01). It would appear that accumulation of QUIS at 200 μM is predominantly via a Na⁺⁻dependent transport site but at higher concentrations (e.g. 2mM), a lower-affinity, Cl⁻-dependent component augments uptake. The influence of medium HCO₃⁻ and Ca²⁺ content on QUIS uptake is presently under investigation.

We thank the Medical Research Council.
516.7

Excessive activation of N-methyl-D-aspartate (NMDA) receptors is thought to mediate neurotoxicity during cerebral anoxia, stroke, and several neurodegenerative diseases. NMDA antagonists therefore have therapeutic potential for these diseases. One type of antagonist (e.g., MK-801) is a use-dependent NMDA open-channel blocker. Recently, memantine, an antiviral and antiparkinsonian drug, was also reported to be a use-dependent antagonist of NMDA-evoked current and to inhibit [3H]MK-801 binding (Bormann, Exp. Brain Res. 1989; 166:591; Kornhuber & Bormann, ibid. 589). We used the whole-cell patch clamp technique on rat retinal ganglion cells to characterize further the mechanism of block of NMDA-activated current. At a holding potential (VH) of -60 mV, 12 μM memantine selectively and nearly completely inhibited the current elicited by 200 μM NMDA (n = 11) without affecting kainate (30 μM) and quisqualate (5 μM) responses. The memantine blockade was voltage dependent (little inhibition at VH = +50 mV) and could be abolished by pre-exposure to 3 mM extracellular magnesium. The rate of recovery from memantine blockade was also voltage- and agonist-dependent. Taken together, the evidence suggests a mechanism of open channel block. Compared to MK-801, however, memantine has much faster micropipet blocking and unblocking rates (+1.3 s and 6.5 s, respectively; VH = -60 mV, KD = 2 μM). With these fast kinetics at concentrations previously shown to be clinically tolerated, memantine-like drugs may eventually prove useful as NMDA antagonists for future therapeutic intervention.

516.8

The endogenous polyamines (PA) spermine and spermidine specifically modulate NMDA-receptor mediated events (e.g. [3H]MK801 binding and ion currents). Antagonists and inverse agonists suspected of interacting with this polyamine site have also been described. We have characterized the activity of various agonists, antagonists and inverse agonists at the NMDA-receptor in a system utilizing NMDA-stimulated changes in intracellular calcium (Ca2+) of cultured chik cortical neurons. Spermine and spermidine potentiated an increase in intracellular calcium in the presence of maximally effective concentrations of NMDA (500 μM) and glycine (100 μM) (EC50s: 346 μM and 734 μM, respectively). Both compounds demonstrated an inversion of the dose response curve for concentrations above 1.5 mM in the presence of NMDA and glycine. Both polyamines were moderately effective at elevating Ca2+ in the absence of added NMDA and glycine (11% of basal) likely due to endogenous glutamate and glycine. Contrary to previous reports, in the present system the purported antagonist diethylthieryamine (DET) and the endogenous PA putrescine were virtually ineffective at the PA recognition site showing only moderate (12%) reversal when administered at 2.5 mM. Arcaine and diaminoephane (DA10) were potent antagonists (250 μM, approx. 40% reduction) of PA-stimulated increases in Ca2+. Arcaine and DA10 also displayed inverse agonist properties in the presence of NMDA and glycine (53% and 38% decrease of maximal NMDA/glycine response, respectively). It is possible that the activity of Arcaine and DA10 in the absence of added PA agonists is due to interaction with endogenous polyamines.

516.9
NON-COMPETITIVE NMDA-CHANNEL BLOCKERS INCREASE MEMBRANE CONDUCTANCE AND GLUTAMATE RESPONSES IN CULTURED ASTROCYTES. C.M. Müller, C. Kom huber & Bormann, Max-Planck Institute for Developmental Biology, Tübingen, FRG; 1 Dept. Neurobiol., University Heidelberg, Heidelberg, FRG

Astrocytes have been shown to express multiple transmitter-gated ion channels, including glutamate-gated kation conductances. We used the whole cell patch clamp technique to study the influence of the non-competitive N-methyl-D-aspartate (NMDA) channel blockers ketamine and MK-801 on glutamate activated membrane currents of cultured rat cortical astrocytes. Application of 300 μM glutamate (10-5-10-4 M) activated an inward current ranging from 50 to 600 pA. Concomitant application of MK-801 or ketamine (10-4-10-3 M) resulted in a pronounced increase (up to 300%) in current amplitude. Application of the NMDA antagonists alone, also elicited a small inward current of both, reversed near 0 mV excluding the possibility that the currents reflect an uptake mechanism. In addition, we also observed a comparable potentiation of kainate currents by NMDA channel blockers. The data show that astroglial cells are targets of non-competitive NMDA antagonists and call for special care in the interpretation of experiments obtained with systemic application of these compounds. Supported by BMFT 316902A5.

516.10
COMPETITION BETWEEN Mg2+ AND PCP FOR BLOCK OF NMDA ACTIVATED CHANNELS IS REDUCED AT DEPOLARIZED POTENTIALS. M.V. Bennet*, A. Garcia-Ballesteros, R.S. Zukin*, and J. Lerma. Instituto de Neurobiología S. Ramon y Cajal, CSIC, Madrid, Spain and Albert Einstein College of Medicine, Bronx, NY 10461

Recent data has suggested that the non-competitive glutamate receptor antagonist ifenprodil, and the inverse agonist diethyltriamine (DET) and the endogenous PA putrescine interact in a competitive manner with spermine at the polyamine site. On the other hand, it has been shown that at concentrations previously reported to be clinically tolerated, ifenprodil greatly impede the ability of phencyclidine (PCP) to block NMDA activated channels. Receptors were expressed in Xenopus oocytes injected with rat brain mRNA and voltage clamped at -60 mV (Lema et al, Neurosci. Lett. 123: 187-191, 1991). Block was competitive in that Mg2+ decreased the potency of PCP in blocking open NMDA channels without changing its maximum effect, i.e., complete block. Channel block by Mg2+ or PCP alone is reduced by depolarization, and since neurons are often depolarized, it was of interest to determine the effect of depolarization on the interaction of PCP and Mg2+. PCP and Mg2+ applied together reduce NMDA-induced currents more than do either blocker alone. However, the degree of PCP block can be estimated by removal of NMDA and blockers, thereby trapping PCP in the channel, and reaplication of NMDA alone, which provides a test of the extent of PCP trapping and of the original degree of block. By this method we found that at -20mV Mg2+ (0.5mM) had almost no effect on PCP block of NMDA-activated channels. This result is not unexpected, given the greater charge on Mg2+ and the likelihood that a substantial component of PCP binding is due to non-electrostatic interactions. Our results suggest that neuronal activity will affect Mg2+/PCP interaction and alter the pharmacodynamics of PCP. This factor may account for differences in behavioral effects of PCP receptor ligands and competitive antagonists at NMDA receptors.
517.1 ALZHEIMER'S AND CONTROL BRAIN CONTAIN SOLUBLE B β AMYLOID PROTEIN PRECURSOR DERIVATIVES EATING AT POSITION 15 OF THE B AMYLOID PROTEIN (APP) DELINEATES THE C-terminal FRACTION containing the full-length APP and APP x 8 fragments. D. Lavery1, M. Usiskin1, B. Wang2, H. Zurcher-Neel2, and S. Younkin1. Case Western Reserve University and the Cleveland Clinic, Cleveland, OH 44106.

To identify the carboxyl terminus of the ~125- and ~105-kDa soluble ß amyloid protein precursor (APP) derivatives produced from full-length membrane-associated APP, we purified these from control human brain, solubilized the CNBr peptides by RPLC using a C18 column, identified putative C-terminal peptides by ELISA with anti-APP C-terminal antibodies generated by us (Pasternack, R. Cotter, R. Gage, B. Greenberg, this meeting). To implement this approach, we first analyzed the abundant soluble APP derivatives secreted by S9 infected cells, using a monoclonal antibody (mAb) recognizing the full-length APP (Bac695), or APP x 8 (Bac751), or APP x 7 (Bac752) of the CNBr peptide maps of the soluble products identified and identified in the CNBr digest of these derivatives, and analysis by direct sequencing, amino acid composition analysis, and western blotting showed the fragments to be, APP x 8 and APP x 7. This indicates that S9 infected cells, like human embryonic kidney (293) cells (Estus et al., Science 248: 1122, 1990), produce soluble derivatives by cleavage of full-length APP at either APP x 8 or APP x 7. It is notable, however, that this is the only or even the major cleavage producing soluble derivatives in human neuroblastoma. Additional analysis is needed to determine if cleavage producing soluble derivatives occurs at multiple sites producing the multiplicity of soluble APP-C-terminal fragments that we have observed in human brain and cultured cells (see Estus et al., Golde et al., these meetings). It will be important, in particular, to determine if even a small fraction of the soluble derivatives in AD or control brain are protein variants that contain the entire APP.

517.2 C-TERMINAL FRAGMENTS CONTAINING FULL LENGTH B AMYLOID PROTEIN ARE PRODUCED DURING NORMAL PROCESSING OF THE B AMYLOID PROTEIN PRECURSOR APP (APP). S. Estus, L. Younkin, M. Usiskin, and S. G. Younkin. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106.

Processing of the APP brain precursor by sequential proteolytic cleavage yielding a soluble C-terminal truncated form and a membrane-associated C-terminal fragment. Using a pulse-chase paradigm followed by immunoprecipitation of cellular lysates with an anti-C-terminal BAPP antibody (provided by D. Selkoe), we studied the processing of the APP in human embryonic kidney cells (293) and Sf9 insect cells (Esch et al., Proc. Natl. Acad. Sci. USA 87: 870, 1990). The cleavage products were detected using Western blot analysis with antibodies against the first 17 aa of the BAPP (provided by B. Greenberg) and 2) coronation of the second largest fragment with a band augmented in 293 cells transfected with constructs expressing the full length APP through the C-terminus of the APP essentially the same set of at least 5 C-terminal fragments is present in control 293 cells (although at a much reduced level); in a variety of human cell lines including fibroblasts, neuroblastomas, and a teratocarcinoma; and in human brain (see Estus et al., these meetings) indicating that APP processing in human brain is similar to that in cultured cells. However, marked quantitative differences in the proportions of these fragments are observed in various cell lines and tissues, and several cell lines produce additional fragments. We are currently investigating if these differences can be identified are generated throughout the secretory pathway that produces soluble BAPP derivatives or via an alternative metabolic route.

517.3 POTENTIALLY AMYLOGENIC C-TERMINAL FRAGMENTS OF THE B AMYLOID PROTEIN PRECURSOR IN HUMAN BRAIN. S. Estus, T. Golde, T. Korenblut, D. Blakesley, M. Eisen, D. Gage, M. Usiskin, B. Greenberg, and S. Younkin. Case Western Reserve University, Cleveland, OH 44106; Nat. Inst. of Neurosciences, Kogenz, Tokyo, Japan; Biological Research Lake, Kalamazoo, MI 49048.

The 38-42 residue ß amyloid protein (MAP) deposited as amyloid in Alzheimer’s disease (AD) is an internal peptide that begins 99 residues from the C-terminus of APP (Pasternack, R. Cotter, R. Gage, B. Greenberg, this meeting). Previous studies in this and other laboratories have established that full-length APP is normally cleaved near its N-terminus to produce large secreted variants and small C-terminal fragments. To isolate BAPP C-terminal fragments from human brain, detergent extracts of a human cerebral cortical membrane fraction (more than 4000 lines from 10 donors less than 50 years old who died of non-neurological causes) were purified using a monoclonal antibody (mAb) recognizing the full-length APP (Bac695), or APP C-terminal derivatives and small C-terminal fragments. To isolate BAPP C-terminal fragments from human brain, detergent extracts of a human cerebral cortical membrane fraction were passed into an immunofinity column containing a polyclonal antibody (anti-APP C-terminal) that was used to isolate and concentrate the APP C-terminal fragments. Following elution from the immunofinity column, the C-terminal fragments were separated using Tris/tricine SDS/PAGE, and each of these proteins was specifically labeled with antibody targeting the recombinant C-terminal peptide of APP. Proteins with antibody targeting the recombinant C-terminal peptide of APP were excised from the Tris/tricine SDS/PAGE gel and processed for mass spectrometry. Each of these proteins was immunoblotted. The superior resolution of small proteins afforded by Tris/tricine SDS/PAGE revealed at least 5 distinct proteins (~11.8, ~11.4, ~10.9, ~9.6, and ~8.7 kDa) migrating between the 6 and 12 kDa markers. Each of these proteins was specifically labeled with antibody targeting the recombinant C-terminal peptide of APP. Taken together, these data indicate that multiple C-terminal MAP fragments are produced in human brain some of which contain the entire MAP and hence are potentially amylogenic. SIGNIFICANTLY, OUR PRELIMINARY DATA INDICATE THAT TISSUES SHOWING HIGHER LEVELS OF APP IMMUNOREACTIVITY, BUT NOT A LOWER LEVEL. WE HAVE LOOKED AT C-terminal MAP fragments in 10 donors less than 50 years old who died of non-neurological causes. Calcium-stimulated MAP immunoreactivity was lower in the young donors. However, 3 of the 3 cells from young donors and all 3 AD cell lines expressed APP at much lower levels than the 3 donors with normal calcium responses. APP was abundantly expressed in 7 of the young donors. However, 3 of the 3 cells from young donors and all 3 AD cell lines expressed APP at much lower levels than the 3 donors with normal calcium responses. APP was abundantly expressed in 7 of the young donors. However, 3 of the 3 cells from young donors and all 3 AD cell lines expressed APP at much lower levels than the 3 donors with normal calcium responses. APP was abundantly expressed in 7 of the young donors. However, 3 of the 3 cells from young donors and all 3 AD cell lines expressed APP at much lower levels than the 3 donors with normal calcium responses.


Several secreted or membrane-bound isoforms of amyloid precursor protein (APP) exist which may contain or lack a Kunitz-type protease inhibitor or an extramain. Using a sensitive protease inhibitor assay as well as an extramain assay of different domains, we have purified isoforms of APP from PC12 cells after induction with bFGF. Biochemical characterization demonstrated differences in the glycoseylation pattern of membrane and secreted APP forms. Furthermore, the major secreted form contained the Kunitz-type protease inhibitor but lacked the extramain. The extramain, however, was present in membrane-bound APP isoforms. As compared to secreted APP, Kunitz-type protease inhibitor containing isoforms were reduced in membrane-bound APP. These biochemical differences may contribute to differences in yet unidentified functions of membrane-bound and secreted APP. Currently we are testing APP isoforms for their effects on growth and survival of distinct CNS neuron types.


Amyloid Precursor Protein (APP) expression was examined in cell lines of olfactory neuroblasts (ON) from human donors. In basal conditions of cell culture, ON protein, and gene expression was found to consist of primarily APP x 770 and APP x 551. PCR analysis found the ratio of APP x 770:APP x 551:APP x 695 is 65:100:7.50. The amount of APP detected by immunoblotting with antibody targeting the APP C-terminal region of the molecule appeared to vary among individuals. Antibodies targeting the N-terminus of APP also detected amino acid sequence variation in APP immunoreactivity, but to a lesser extent. We have looked at C-terminal MAP fragments in 10 donors less than 50 years old who died of non-neurological causes. Calcium-stimulated MAP immunoreactivity was low in 7 of the young donors. However, 3 of the 3 cells from young donors and all 3 AD cell lines expressed APP at much lower levels than the 3 donors with normal calcium responses. Calcium-stimulated MAP immunoreactivity and resulted in approximate equalization of APP levels for all cells. Because immunoprecipitation experiments showed calcium-induced decrease (rather than increase) in APP levels, we suspect that the apparent calcium-induced increase in APP levels seen by immunoblotting may be an artifact of calcium-induced modification of APP. Analysis of APP expression in a non-neuronal cell model (lymphoblasts) did not indicate any variation in baseline or calcium-stimulated MAP levels above AD levels and controls.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991

S17.7
PLASMA AMYLOID PRECURSOR PROTEIN IS A MARKER FOR ALZHEIMER'S DISEASE. A.I. Bush*, B.D. Moss*, Q.L. Li,
J. Gurrola, J. Martin, C. Masters. Dept. of Pathology, Univ. of Melbourne, Parkville, VIC, Australia and # Center for Molecular Biology, Univ. of Heidelberg, Heidelberg, Germany.

5A4 amyloid deposition in brain, characteristic of Alzheimer's disease (AD), may reflect abnormal accumulation of the amyloid precursor protein (APP) and should be correctly processed. A blood marker reflecting this abnormal metabolism would be of predictive and diagnostic value, as well as provide a means of monitoring the efficacy of therapeutic interventions. We have previously sequenced APP from the human platelet where it is detected by Western blotting as 130 and 110 kDa proteins and may be released with the 5A4 domain intact. Normal control plasma forms of 65 and 42 kDa. We analysed immunoblots of plasma APP from AD cases (n=26) and controls (n=34) and found a 60% increase of the 130 kDa species of APP in AD (p<0.001), no differences in 110 and 65 kDa forms and a 35% decrease in the 42 kDa form (p<0.001). Studies of protease action on APP indicated that plasma APP forms may be proteolytic cleavage products. The APP abnormalities in the circulation in AD may reflect a failure of constitutive proteolysis due to modifications of APP, or anomalies involving degradative pathways, co-factors or inhibitors. Abnormalities of constitutive APP proteolysis may allow APP to be processed through an alternative, amyloidogenic pathway. Our studies show that the source of plasma APP and the mechanism responsible for the APP differences detected may lie outside the vascular compartment.


S17.8
FORMS OF B-AMYLOID PRECURSOR PROTEIN (β-APP) IN HUMAN PLATELETS AND LYMPHOCYTES. M. Schlossmacher*, B. Ostaszewski*,
Liesebach*, K. Kose, D. Selko, Brigham & Women's Hosp., Harvard Medical School, Boston, MA 02115;+Athena Neurosciences, San Francisco 94080.

β-amyloid protein (APP) is progressively deposited in cerebral plaques and meningeocerebral vessels in Alzheimer's disease (AD). AD shares numerous features with amyloidoses of circulating origin. The soluble, β-terminated ~125 kDa form of βAPP (i.e., βAPP), which we have identified in plasma, has also been localized to platelet α-granules. Full-length (FL) βAPP has been reported to be released from agonist-stimulated platelets as membrane microparticles. We have examined peripheral blood cells with regard to βAPP isoforms. After purification of platelets from contaminating leukocytes, we detected the mature, N- plus O-glycosylated ~ 40 kDa FL-βAPP in platelet membranes but only trace amounts of immature, N-glycosylated βAPP. In the cell-free release of activated platelets, we found abundant βAPP but failed to detect FL-βAPP associated with microparticles. Membrane extracts of resting and stimulated platelets also revealed comparable amounts of a ~ 10 kDa fragment, which probably represents the C-terminal portion of βAPP after cleavage of βAPP.

2. B and T lymphocytes isolated from peripheral blood and cultured EBV-transformed B cells contained small amounts of internal membrane-associated mature and immature FL-βAPP. The presence of amyloidogenic FL-βAPP molecules in peripheral blood cells has potential relevance for the pathogenesis of Aβ deposition in AD.

S17.9
PROCESSING OF β-AMYLOID PRECURSOR PROTEIN IN ASTROCYTES AND MICROGLIA FAVORS A LOCALIZATION IN INTERNAL VESICLES. G. Haass, A. Hung* E.H. Koo, and D.J. Selkoe. Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston MA.

We report the expression of βAPP in microglial cells and astrocytes in primary cultures of cerebral cortex of newborn rats. Both cell-types express substantial amounts of βAPP 695, 751 and 770 as determined by metabolic labeling followed by immunoprecipitation with anti-βAPP monoclonal antibodies. In the cell-free releasate of activated platelets, we have identified βAPP in G0-like structures, with very little cell surface staining. From these data, we propose that at least in astrocytes and microglia cells, βAPP may primarily occur in an intracellular, membrane-associated form rather than being cleaved and secreted or inserted at the cell surface. It may thus perform a principally intracellular function. We also present preliminary data showing that microglial cells secrete C-terminal-containing βAPP fragments into the media. These fragments are potentially amyloidogenic since they contain the C-terminus and β the peptide epitopes.

S17.10

The β amyloid protein causes neuronal degeneration in primary rat hippocampal neurons after 3 days in culture (Yankner et al. Science 250:279, 1990). We have assayed for neuronal expression of the neurofibrillary tangle-associated Alz-50 immunoreactive proteins (Alz-50 i.p.s.) by quantitative immunoblot analysis and immunocytochemistry. The Alz-50 i.p.s. are expressed at low levels during neuronal development in primary hippocampal cultures as two isoforms with molecular weights 55 and 68 KD. Treatment with β amyloid induced a 4–5-fold increase in Alz-50 i.p.s. which was not observed after addition of control peptides or a reverse sequence β amyloid peptide. The levels of several other cytoskeletal proteins were unchanged. The β amyloid dose response for induction of Alz-50 i.p.s. was quite similar to the dose response for the neurotoxic effect. Treatment with the excitoxic glutamate did not induce the Alz-50 i.p.s. In addition, co-cultivation of β amyloid with the excitatory amino acid antagonists kynurenic acid and APV did not affect the induction of Alz-50 i.p.s. Preincubation of neurons with cycloheximide prevented subsequent induction of Alz-50 i.p.s. by β amyloid. Thus β amyloid can specifically induce the altered cytoskeletal proteins associated with Alzheimer's disease by a mechanism which requires protein synthesis and which differs from that of excitatory amino acids.

S17.11

The role of β amyloid protein in the pathogenesis of neuronal degeneration in Alzheimer's disease (AD) is a central issue in the illness. We found that focal deposition of β amyloid in adult rat cerebral cortex causes profound neurodegenerative changes including neuronal loss, degenerating neurites and inclusion of Alz-50 immunoreactive proteins associated with AD. In the present study we examined the effects of local or systemic administration of substance P on the neurodegenerative effects. β-amyloid (3 nmol) or control peptides were stereotactically injected into parietal cortex in a volume of 1 μL. Animals were sacrificed at 1 week and perfused for histologic evaluation. Neuronal counts were made in a 0.64 mm2 square in the area of maximal neuronal degeneration. Coinjection of substance P (2-200 pmol) with β amyloid dose-dependently prevented neuronal loss. Induction of Alz-50 immunoreactive proteins detected by either immunocytochemistry or immunoblot analysis, was also blocked by substance P. Peripheral administration of substance P in a dose of 2-200 nmol/kg i.p. 30 min before intracortical injection of β amyloid dose-dependently blocked the neurotoxicity. Administration of substance P for up to 24 hr after the β amyloid injection was equally protective, but the effect dissipated by 72 hr. These results show that β amyloid neurotoxicity in vivo is prevented by either local or systemic administration of substance P.

S17.12
EFFECTS OF BETA AMYLOID III: THE PRIMATE BRAIN A.C. McKee, N.W. Kowall, M.F. Beghi, J. Schumacher and B.A. Yankner. Dept. of Neuropathology, Neurology and Neurosurgery, Massachusetts General Hospital, Children's Hospital and Harvard Medical School, Boston, MA 02114

Deposition of β amyloid protein in the brain is a universal feature of Alzheimer's disease (AD). To determine the effects of β amyloid deposition, we performed intracerebral microinjections of β amyloid peptide (40D) in cynomolgus monkeys. Bifrontal craniotomies were performed with the resection of a dural flap. Microinjections of 61-42 and control peptides were performed under stereotoxic guidance. After survival intervals, the brains were perfusion-fixed, cryoprotected and cut at 50 μm on a freezing microtome. Sections of serial sections were processed for histopathology and immunocytochemistry. The monoclonal antibody Alz 50, and antibodies to tau, B amyloid and ubiquitin. The 61-42 peptide injections produced a defined 61-42 amyloid-immunoreactive deposit surrounded by neuronal degeneration and abnormal ubiquitin and Alz 50 positive neurites. The extent of degeneration was dose-dependent.

Control peptides produced cortical disruption at the injection site without a significant lesion. A substituted 61-40 peptide produced a smaller lesion than that caused by native 61-40. These findings suggest that β amyloid protein is neurotoxic and may, therefore, be responsible for neuronal degeneration in AD.
PIGMENTED NEURONS IN THE SUBSTANIA NIGRA OF THE
GANGLIOSIDE GM1 MAY COUNTERACT THE MPTP-INDUCED
PIGTAIL MACAQUE.1A M Tanson.

Two female pig-tailed macaques (b.wt. 5.9 kg) were infused with l-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 2 mg/kg) in the left internal carotid artery. Starting 30 min after MPTP infusion one macaque was treated with the ganglioside GM1 (20 mg/kg) daily, i.m. for 18 days. The other macaque received saline injections. The day after the last GM1 injection the animals were sacrificed, perfused with 4% paraformaldehyde, and processed for immunohistochemistry after cryostome sectioning of the entire substantia nigra (35μm coronal sections). After Nissl staining the total number of pigmented neurons in the substantia nigra were estimated unbiased using the optical dissector (Olympus) and the total volume of nigral side, 13,200 μm

The degree of rigidity was milder in the GMI-treated animal, which sometimes used 1,2,3,6-tetrahydropyridine (MPTP) 2 mg/kg in the internal carotid artery.

The degree of rigidity was milder in the GMI-treated animal, which sometimes used thioflavin-saline treated macaque. The unbiased estimates of the total number of pigmented neurons in the substantia nigra were: saline treated animal: 89,000 (unlesioned side); 42,000 (lesioned side); GM1 treated animal: 101,000 (unlesioned side); 38,000 (lesioned side). The estimates of the mean volume were: saline treated animal: 2.9,000 μm (lesioned side); 7,400 μm (unlesioned side); GM1 treated animal: 10,800, μm (lesioned side), 13,200 μm (lesioned side).

This pilot study gives support for the previously observed effect of GM1 to counteract the MPTP-induced shrinkage of nigral tyrosine hydroxylase immunoreactive neurons in the male C57BL/6J mouse. Thus, ganglioside GM1 may exert anti-parkinsonian actions.

EYE EXTRACT COMPONENTS PROMOTE ACETYLCHOLINE SENSITIVITY IN CHICK DORSAL ROOT GANGLION NEURONS. J. P. Margiotta. Dept. of Physiol. & Biophysics., Mount Sinai School of Medicine, NY, NY 10029.

Eye tissue extract contains at least two activities that support the survival and development of chick ciliary ganglion neurons in culture: a neurotrophic activity and a 50 kDa factor that increases acetylcholine (ACH) sensitivity. Recent studies indicate that the neurotrophic (growth promoting) activity can substitute for nerve growth factor (NGF) in supporting short-term survival of chick dorsal root ganglion (DRG) neurons (Eckenstein et al., Neuron 4:623-35, 1990), and that DRG neurons express both ACh receptor (AChR) mRNA and functional nicotinic AChRs (Boyd et al., J. Neurobiol. 22: 1-14, 1990). In order to examine factors influencing the appearance of functional AChRs on sensory neurons, embryonic day 13 (E13) DRG neurons were grown in basal culture medium supplemented with either NGF (10 ng/ml) or eye extract (3% v/v) for up to 7 days, and examined for responses to 500 μM ACh using whole-cell recording methods. Close to 100% of the cells identified as DRG neurons after 1d in NGF and eye extract-supplemented culture media survived for 6 days, while <50% of neurons in basal medium survived. After 5-6 d, 95% of the neurons grown with eye extract displayed ACh responses well above a criterion level of 2 pA/μm² (geometric mean response=12±2 pA/μm², n=35) while only about 50% of the neurons grown in basal media alone or with NGF did so, and the responses were much smaller [3±1 pA/μm² (n=20) and 3±1 pA/μm² (n=25), respectively]. At an equivalent developmental age (E18,19) 95% of freshly dissociated DRG neurons were sensitive to ACh, identical to the proportion seen after 6d in culture media containing eye extract, and representing a >2-fold increase from the proportion seen for neurons obtained at E13,14 (n=18). These results indicate that eye extract components, different from NGF, promote the ACh sensitivity of DRG neurons in culture, and suggest that such factors may influence AChR appearance in vivo.

Supported by NIH NS24417 & March of Dimes 5-742.

IBOTERIC LEISON IN STRIATUM TRIGGERS EXPRESSION OF NEW AND INCREASES FIVE EXISTING PROTEINS. F.C. Zhou, Y. Kim, S. C. Fu, and B. S. Kwon. Departments of Anatomy and microbiology, Indiana Univ. Sch. Med. Indianapolis, IN, 46202, USA.

Neuronal toxin, ibotenic acid (IB), injection caused neuronal degeneration and gli activation and proliferation in the adult rat brain. The region of non-neuronal and reactive glial environment was subsequently heavily invaded by serotonergic components, different from NGF, promote the ACh sensitivity of DRG fibers beginning three days and prevailed after 20 days. Such an environment also fostered a high survival rate of transplanted fetal neurons as compared to normal IB-lesioned animals. A protein/peptide of 45KD newly appeared in the 8-day-post-IB-lesion animals. A protein/peptide of 45KD newly appeared in the 8-day-post-IB-lesion animals. A protein/peptide of 45KD newly appeared in the 8-day-post-IB-lesion animals.


2 Insulin-like growth factors (IGFs) are potent mitogenic and differentiating promoters of many developing tissues including brain. To determine the role of IGF I on the differentiation of DA neurons in the developing mesencephalon, we have been studying the influence of primary DA neurons cultured in serum free medium. Mesencephalon from 14 d rat embryos were dissociated and cultured in 10% FCS for 48 hours, then maintained in SFM or without IGF I. At 7 days in vitro, H3-dopamine total uptake, K+ stimulated release were measured. Total uptake of H3-dopamine was increased from 861 dpm/min (SFM) to 1334 dpm/min (IGF I 100ng/ml), (p<0.01) indicating an unexpected capacity for uptake and storage of neurotransmitter with IGF I treatment. K+ stimulated release of H3-dopamine was increased significantly (p<0.05) from 419 dpm/min to 715 dpm/min (IGF I 50ng/ml). Ca2+ stimulated release of H3-dopamine was not significantly altered upon treatment with IGF I. Immunocytochemistry with antisera against tyrosine hydroxylase and morphometric measurements of the identified neurons demonstrated a significant increase in the soma size, and in the number and length of neurite processes, following treatment with IGF I. We conclude that IGF I promotes uptake, storage and K+ mediated release of H3-dopamine, as well as neuronal morphology of DA neurons in vitro.

Supported by (MRC and Dept. Peds., UWO)

Delayed delivery of a cortically derived trophic factor rescues axotomized, slowly-degenerating neurons of the dorsal lateral geniculate nucleus (DLCN) and improves visual discrimination. F. Haun, T. Jaret and T.J. Cunningham, Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

Visual cortex lesions in adult rats lead to cell death in two populations of dLGN neurons; in one population (generated late in neurogenesis) loss is maximal by 2wks. post-lesion, while slowly generated neurons a similar maximal loss is not reached until 2mos. post-lesion. Using an implanted osmotic minipump, we delivered a 2-week supply of a neuron survival factor to the visual cortex lesion site beginning 2wks. post-lesion, and tested the animals behaviorally starting 2mos. post-lesion. This factor was derived from an HPLC fraction of culture medium conditioned by embryonic occipital cortex explants. Animals receiving the factor learned a difficult visual discrimination more than twice as fast as animals with comparable lesions that received a control fraction of unconditioned culture medium. 3H-thymidine counts of labeled dLGN neurons showed that the factor rescued all neurons that otherwise die between 2wks. and 2mos. post-lesion. These results show that delayed delivery of a trophic factor following CNS injury can nonetheless rescue slowly degenerating neurons, with a resulting behavioral improvement. Supported by NIMH grant MH44734 (FII) and NIH grant NS15487 (TJC).

A factor in conditioned medium from retinal pigment epithelium (RPE) mediates photoreceptor survival in vivo. H. Gauder, A.J. Sweett and J.E. Turner. Departments of Neurobiology and Anatomy and Ophthalmology, Bowman Gray School of Medicine, Winston-Salem, NC.

Maintenance of visual gene expression in photoreceptor cells (PRC) of dystrophic RCS rats results in sprouting of transplants of retinal cells in the RCS retinas have been studied by immunofluorescence (Exp. Eye Res., 1991). These studies indicated that a PRC secreted factor mediated PRC rescue. We have used an in vitro PRC culture system to characterize this factor. We report PRC survival and neurite promotion by medium conditioned by cultured normal PRC (RPE-CM). Up to 87% of cells expressed immunocytochemically detectable opsin in RPE-CM. In RPE-CM from RCS retinas only 14% cells expressed opsin. Analysis of basic fibroblast growth factor (bFGF) levels (shown effective in achieving PRC rescue in vivo) in RPE-CM from normal and dystrophic RCS retinas indicated no significant differences by ELISA. Analysis of metabolically labeled proteins in normal and dystrophic RPE-CM indicated specific differences. Efforts are currently underway to test various components of the RPE-CM for PRC rescuing abilities.

Supported by a grant from NIH (EY-04377) to JET.

Insulin-like growth factor-I enhances secretion from chromaffin cells and that E-protein kinase C may be the source of STRI culture.

Medicine, Med. Sch., Nagoya City Univ., Mizuiho-ku, Nagoya, Japan 467 and Res. Inst. Sumitomo of synthetic de-acetylated HCNP (free-HCNP) were treated cells was reduced to that seen in untreated cells when the cells were incubated normally seen in IGF-I treated cells. High K+-stimulated release of stored H3-dopamine was increased significantly (p<0.05) from 419 dpm/min to 715 dpm/min (IGF I 50ng/ml). Ca2+ stimulated release of H3-dopamine was not significantly altered upon treatment with IGF I. Immunocytochemistry with antisera against tyrosine hydroxylase and morphometric measurements of the identified neurons demonstrated a significant increase in the soma size, and in the number and length of neurite processes, following treatment with IGF I. We conclude that IGF I promotes uptake, storage and K+ mediated release of H3-dopamine, as well as neuronal morphology of DA neurons in vitro.

Supported by (MRC and Dept. Peds., UWO)
1 AUTORADIOGRAPHIC DISTRIBUTION OF CHOLINERGIC MUSCARINIC RECEPTORS IN OLFACTORY BULBECTOMIZED (OB) RATS AFTER CHRONIC TREATMENT WITH MIANSEERIN AND DESIPRAME.
519.7

Gene expression of choline acetyltransferase (CHAT) in the central nervous system was studied by use of in situ hybridization histochemistry. RNA probes against CHAT templates were synthesized using in vitro transcription in the presence of digoxigenin-11-UTP. The probe was detected by an antibody against digoxigenin coupled to alkaline phosphatase. This method resulted in a large number of cell bodies and occasional proximal processes being intensely labeled against a virtually undetectable background. Telencephalic cells expressing CHAT mRNA were found in the following striatal and basal forebrain nuclei: the caudate-putamen, nucleus accumbens, olfactory tubercule, medial septal nucleus, vertical and horizontal diagonal band nuclei, substantia innominata, and nucleus basalis. Cortical cells were not labeled with this probe. In the diencephalon, only cells of the medial habenula were labeled with the CHAT mRNA probe, other thalamic areas and the hypothalamus did not contain labeled cells. In the brainstem, only the pedunculopontine and laterodorsal tegmental nuclei and motor cranial nuclei 3, 4, 5, 7, 9, 10, and 12 contained cells labeled with the CHAT mRNA probe. In the spinal cord, dorsal horn neurons exclusively exhibited CHAT mRNA label. The distribution of CHAT mRNA labeled cells in this study corresponds conservatively to previously identified cholinergic cells. [Support: USPHS grant NS 10928 to L.L.B.].

519.8
THE RAPID KINETICS OF ACETYLCHOLINE RELEASE FROM RAT BRAIN SYNAPTOSOMES: ANALYSIS BY RAPID SUPERFUSION. L. B. Pearce and E. Adams* Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Spontaneous and evoked [3H]ACh efflux from rat brain synaptosomes was investigated on the second and millisecond time scale using the technique of rapid superfusion. Synaptosomes, radiolabeled by preincubation with [3H]ACh, were superfused with Krebs-bicarbonate buffer, pH 7.4 in 0.3 to 0.5 ml/second. The mixing half-life was 119 milliseconds (ms) and efficiency of superfusion > 85%. Superfusion for 48 seconds with isosmotic Krebs buffer resulted in 1.57, 5.68, 7.19, 8.80, and 18.32 percent release of tritium in the presence of 10, 20, 30, 50, 75, and 100 mM potassium ion, respectively. Kinetic analysis of net potassium-stimulated release revealed a single component of release that fit a single exponential function with a t<sub>1/2</sub> = 12.7, 13.1, 15.4, and 12.7 seconds for 20, 50, 75, and 100 mM KCl. Analysis of the area under the tritium efflux curves observed for serial collection of 50 ms samples over 750 ms revealed that 0.111, 0.550, 0.614 % net tritium release was evoked by superfusion with isosmotic buffer containing 20, 50, and 100 mM KCl, respectively. Potassium-evoked release observed on both the second and millisecond time scales was totally calcium-dependent whereas, only a small fraction of spontaneous release was dependent on calcium. This work was supported by a grant from The Epelie Foundation for Research.

519.9

Military Performance and Neuroscience Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760.

Hippocampal muscarinic receptors belong to two main pharmacologic subtypes, M<sub>1</sub> and M<sub>2</sub>. The majority of the latter subtype is predominantly located presynaptically, where these receptors could modulate acetylcholine (ACh) release by functioning as inhibitory autoreceptors. M<sub>1</sub> receptors may inactivate this receptor, leading to increased ACh release. The purpose of this study was to assess changes in extracellular ACh in vivo microdialysis following administration of various M<sub>1</sub> antagonists. Microdialysis guide cannulae were implanted into the CA1 hippocampal region of male Fischer rats. After a minimum of two days to recover from surgery, rats were trained to press a lever while chronically microdialyzed. Drug or placebo was perfused via the microdialysis probe. High performance liquid chromatography with electrochemical detection was used for online analysis of the dialysates. M<sub>1</sub> antagonists significantly enhanced ACh release in a dose-dependent fashion as compared to placebo. The present data corroborate studies which show increased ACh levels in vivo following application of M<sub>1</sub> antagonists.

519.10
DOPAMINERGIC REGULATION OF STRIATAL ACETYLCHOLINE RELEASE: IMPORTANCE OF D1 AND NMDA RECEPTORS. G. D. Thanos* and J. A. Butcher. Dept. Psychology and Neurology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

The dopaminergic regulation of striatal cholinergic activity was studied using in vivo microdialysis to measure the intrastriatal concentration of acetylcholine (ACh) and choline in the striata of freely moving rats. d-Amphetamine (2 mg/kg, s.c.) and nomifensine (5 mg/kg, s.c.) increased the concentration of ACh in the striatal dialysate by 40-60%. The selective D1 receptor antagonist SCH 23390 (0.3 mg/kg, s.c.) decreased the concentration of ACh in the striatal dialysate by 13-20%, while the selective D2 antagonist raclopride (1 mg/kg, s.c.) increased striatal ACh release by 30-60%. Raclopride blocked the increase in locomotor activity produced by d-amphetamine but did not interact with the stimulant to further enhance ACh release. In contrast, SCH 23390 completely antagonized both the increase in locomotion and the enhancement of striatal ACh release produced by d-amphetamine. SCH 23390 also blocked the raclopride-induced increase in ACh overflow. These results indicate that d-amphetamine increases ACh release in the striatum via a D1 receptor mechanism. Consistent with this hypothesis, the selective D1 receptor agonist CY 208-243 (1 mg/kg, s.c.) increased striatal ACh release by approximately 60%. Inclusion of the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 in the striatal perfusion solution significantly attenuated the increase in striatal ACh release produced by CY 208-243. Consequently, increased striatal ACh release produced by enhanced D1 receptor stimulation appears to be mediated, at least in part, by activation of striatal NMDA receptors.

519.11

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During REM sleep ponto-geniculo-occipital (PGO) waves are generated by cholinergic neurons in the peribrachial pons. We report here the effect of cholinergically stimulating this brain region. Five cats were chronically prepared for recording wake-sleep parameters. After recovery, carbachol (4.0 µg/250nl) was microinjected into the peribrachial PGO bursting cell zone. Carbachol produced an immediate onset of state-independent PGO waves lasting 4 days and the duration of state-dependent PGO wave was maintained high during a threefold increase in REM. REMN also remained elevated for 6 days (22.2 ± 5.2%, p < 0.001) compared to control values (13.0 ± 3.2%). Beginning on day-2, increased PGO activity was associated with an increase in PGO activity. We postulate that the immediate and sustained increase in PGO activity is due to cholinergetic activation by carbachol of the PGO burst cells and the delayed, sustained increase in REM is a secondary consequence of membrane excitation. Supported in part by NIH grant MH-13973.
520.1 MOLECULAR CHARACTERIZATION OF A STRIATAL ENRICHED PROTEIN TYROSINE PHOSPHATASE

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Modulation of tyrosine phosphorylation is one mechanism for regulating cellular differentiation controlled by opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). A number of PTPases have been cloned and their amino acid sequences determined. Two classes have been identified. The first class is intracellular and the second class has extracellular regions linked to cytoplasmic, catalytic domains through single transmembrane segments. Northern analyses of different tissues indicate wide variability in the regional expression of PTPases, none have been nervous system specific.

Here, we report the isolation and characterization of a cDNA clone which encodes for a PTPase found predominantly in the brain. Within the brain, it shows further tissue specificity by appearing to be a Striatum Enriched Phosphatase and has been designated Step. This new PTPase contains regions homologous to ones found in previously characterized PTPases, and appears to be a distinct member of the cytoplasmic class of PTPases. The deduced amino acid sequence predicts a protein of approximately 369 amino acids. In vitro translation produces a protein with an apparent molecular weight of 46 kDa. Northern analyses of various regions of rat brain reveal a 3 kb and a 4.4 kb mRNA. In this study, the authors present the isolation and characterization of a cDNA clone which encodes for a protein tyrosine phosphatase found predominantly in the brain. They describe the protein's amino acid sequence and its expression patterns in different brain regions.

520.2 TIME-COURSE OF PEPTIDE ALTERATIONS IN STRIATAL EFFERENT NEURONS AFTER UNILATERAL CORTICAL LESION BY THERMOCOAGULATION


We have previously reported (Cheslak and Sakin, Neurosci. Abstr. '90) a bilateral increase in the levels of mRNAs encoding enkephalin (ENK) and substance P (SP), two neuropeptides present in striatal efferent neurons projecting to the globus pallidus (GP) and entopeduncular nucleus, respectively. Here, Northern analyses of different tissues indicate wide variability in the regional expression of PTPases, none have been nervous system specific.

Here, we report the isolation and characterization of a cDNA clone which encodes for a protein tyrosine phosphatase found predominantly in the brain. Within the brain, it shows further tissue specificity by appearing to be a Striatum Enriched Phosphatase and has been designated Step. This new PTPase contains regions homologous to ones found in previously characterized PTPases, and appears to be a distinct member of the cytoplasmic class of PTPases. The deduced amino acid sequence predicts a protein of approximately 369 amino acids. In vitro translation produces a protein with an apparent molecular weight of 46 kDa. Northern analyses of various regions of rat brain reveal a 3 kb and a 4.4 kb mRNA. In this study, the authors present the isolation and characterization of a cDNA clone which encodes for a protein tyrosine phosphatase found predominantly in the brain. They describe the protein's amino acid sequence and its expression patterns in different brain regions.
This study was designed to explore in detail the organization of these projections, and LIMBIC INTERACTION IN THE ROSTRAL CAUDATE NUCLEUS: A. Rosell*, S. de las NEUROANATOMICAL STUDY IN THE CAT.

ROSTRAL CAUDATE NUCLEUS: A. Rosell*, S. de las A NEUROANATOMICAL STUDY IN THE CAT.

next, we microinjected horseradish peroxidase (HRP-WGA) in different portions of the rostral caudate nucleus. Next, we microinjected horseradish peroxidase (HRP-WGA) in different portions of the rostral caudate nucleus. Injections produced a dose-dependent, significant increase of perfused and labeled, HRP injections dose-dependently reduced the HRP-induced dyskinesias. These results indicate that 1) an impairment of corticostriatal GLU transmission can produce oral dyskinesias; 2) a concomitant blockade of nigrostriatal DA transmission can counteract this effect. The implications of these findings for normal brain function and mechanisms of oral dyskinesias are means will be discussed. (Supported by the Ontario Rite Schizophrenia Research Fund and the Research Association of the Ontario Mental Health Foundation).


Haloperidol (0.5 mg/kg/d14), a neuroleptic associated with extrapyramidal syndromes (EPS) and tardive dyskinesia (TD), blocks dopamine (D)-2 receptors, while SCH 23390 (1 mg/kg/d14) blocks DA D-1 receptors. These drugs cause an increase in striatal DA D-2 and D-1 receptors, respectively, and in the percentage of perforated synapses. Administration of haloperidol and SCH 23390 for 14 days prevented the increase in striatal perforated synapses while the DA D-2 and D-1 receptors were increased to the same level as if administered separately. We have shown that the perforated synapse originates from motor cortex and speculate that the increase is due to activation of the corticostriatal pathway. It has been suggested that many striatal neurons possess separate DA D-2 and D-1 receptors. The D-1 pathway projects to the thalamus via the entopeduncular nucleus and the D-2 pathway projects to the thalamus via the globus pallidus, subthalamic nucleus, and substantia nigra zona reticulata. Simultaneous blockade of both receptor subtypes with haloperidol and SCH 23390 could up-regulate the two DA receptors, while converging input to the thalamus could eventually decrease activation of the corticostriatal pathway. Administration of SCH 23390 could be effective in reducing the incidence of EPS and TD associated with haloperidol. Supported by the Dept. of Veterans Affairs and NIMH.


The limbic component of the basal ganglia has been mainly assigned to the so-called ventral striatum. The ventral striatum has, however, a remarkable interconnection with limbic territories located in the medial wall of the cerebral cortex (Powell et al.,1974). This study was designed to explore in detail the organization of these projections, and their role in the limbic circuitry. The study was performed with two different anatomic models: the thalamus and the substantia nigra. Eleven cats were deeply anesthetized and stereotaxically injected with horseradish peroxidase (HRP) and/or fluorogold (FG) as a retrograde tracer. Our data suggest a functional segregation between inter-striatal connections and the ventrolateral striatum may occur through the inputs and outputs of the substantia nigra. The compartmentalization of different projection systems and neurochemical markers into well demarcated striosomes and matrix regions is an established feature of the mammalian neostriatum. The observation of distinct types of neuroactive substances, receptors and their interactions in strionsomes and matrix suggests a functional segregation between these compartments. The present study was undertaken to determine the organization of efferent projections from each of the neostriatal compartments to the entopeduncular nucleus (EP), one of the major output centers of the striatum. Fluorogold, a fluororescent retrograde tracer, was injected into the lateral habenula (LH) or the ventral lateral nucleus of the thalamus (VLT) of adult Wistar rats to identify the topographical organization of the projection neurons projecting to these two major output centers of the EP. In contrast, fluorogold was placed into the rostral or caudal region of the EP, identified from the above experiment as areas that project to the LH or VLT respectively. The fluorescent retrograde labeling was combined with immunocytochemistry for calbindin, a specific marker for the projection neurons of the matrix. The results reveal that the rostral part of the EP projects to the LH and receives input exclusively from the strionsomes, whereas the caudal part of the EP projects to the VLT and receives input exclusively from the matrix. These observations were substantiated by the finding that calbindin immunoreactive fibres lie in relation to those EP neurons that project to the VLT. The data suggest that the strion is involved in a pathway through EP, LH and the dorsal raphe nucleus which in turn projects to the neostriatum, while the matrix is involved in a pathway through EP and VLT which projects to the cerebral cortex.

PHARMACOLOGICAL ACTIVATION OF DOPAMINERGIC PATHWAYS IN THE BABOON STUDIED WITH PET. C.C. Rose* and J.S. Perlmutter. Washington University School of Medicine, St. Louis, MO 63110.

We developed an in-vivo method to measure regional cerebral blood flow (rCBF) responses to dopamine receptor manipulation in the baboon using [123I]iodoamphetamine (IMP) and [123I]iodo- and implanted skull cap that locks into a head holding device equipped with 3 orthogonal N-shaped fiducial markers for PET and MRI was employed for head fixation. Comparison of PET fiducial measurements to those from an MRI obtained in the same plane in each animal permitted precise anatomical identification of rCBF responses. Quantified CBF studies were performed using the bolus O-15 water method and regional responses were identified in 12 different regions of interest before and after I.V. drug administration. We found as much as a 25% reduction in CBF in the lentiform nucleus and thalamus after i.v. injection of the selective D2 agonist against dopamine. These reductions were observed in a range of 0.002 to 0.02 mg/kg and were reproducible within and across different animals. Furthermore, different regional responses occurred for the D1 agonist SKF 38393. Functional effects of specific dopamine pathways manipulation can be demonstrated in-vivo in primates with PET.

OF L-DOPA IN PATIENTS WITH BASAL GANGLIA DISEASE. V. J. Brown, with the response, but when the stimulus-response (S-R) relationship is arbitrary, it was symbolically cued.

In one task (S-R compatible), the stimulus was to the

as possible following a cue. In one task (S-R compatible), the stimulus was to the left or the right. In the other task (arbitrary S-R) the side of the required response was symbolically cued.

Performance of patients with idiopathic Parkinson's disease was compared with that of patients with Parkinsonian-like symptoms which were not responsive to L-dopa. Both groups were tested with and without L-dopa on each type of choice reaction time task. Only the patients with Parkinson's disease were differentially impaired in the two tasks, but it was the task with high S-R compatibility which was more impaired and not the task with an arbitrary S-R relationship. The differential deficit was ameliorated with L-dopa treatment. These results demonstrate a task specific impairment in reaction time performance with differential response to dopaminergic therapy.

21.1 BLOCKADE OF ADENYLATE CYCLASE INCREASES THE RATE OF SYNAPTIC DEPRESSION IN SYPHON SENSORY NEURONS IN APLYSIA. B.A. Goldsmith and T.W. Abrams. Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

We have found that an inhibitor of adenylate cyclase, 9-(tetrahydro-2-furyl)adenine (THFA or SQ 22,536) effectively blocked cAMP-mediated effects produced by 5-HT in siphon sensory neurons (SNs) including spike broadening, reduction of accommodation, and reversal of depression at SN synapses. We observed that synaptic depression occurred more rapidly in the presence of THFA. When SNs were stimulated at an ISI of 15 s, the second EPSP was 35 ± 6% (± SEM) of initial amplitude in THFA vs. 71 ± 6% in controls (p < .001). After 10 SN action potentials were elicited, EPSPs still showed significantly more depression in THFA experiments (29 ± 6% vs. 42 ± 3% in control, p < .001). In addition to acting as a cyclase inhibitor, THFA produces a modest broadening of SN action potentials, possibly by blocking K+ channels. Comparable broadening of the SN APs by the K+ channel blocker 3,4-DAP had no effect on the rate of synaptic depression. These results suggest that cAMP plays an important role in maintenance of normal levels of synaptic transmission.

21.2 POSSIBLE MOLECULAR BASIS OF THE ORDER REQUIREMENT FOR CS-US PAIRING DURING CONDITIONING: BACKWARDS PAIRING OF C34


During classical conditioning of the gill and siphon withdrawal reflex of Aplysia, Ca2+/calmodulin-sensitive cyclase serves as a molecular sieve of convergence within siphon sensory neurons for C34 and serotonin, the cellular representations of the conditioned and unconditioned stimuli (CS and US). Using a perfused membrane cyclase assay, we previously found that a prepulse of C34 resulted in both a faster rate of cyclase activation and greater peak activation by a brief pulse of 5-HT than did a backwards pulse of C34. These results suggested that Ca2+/calmodulin binding to the cyclase might enhance the rate of activation by receptor and Gs. If Ca2+/calmodulin binding influences the rate of Gs-cyclase coupling, we hypothesized it might also affect the rate of Ca2+/cyclase uncoupling once transmitter leaves the receptor. To test this possibility, we measured the time course of cyclase deactivation, in the presence and absence of a pulse of Ca2+ delivered after a transient exposure to 5-HT. The Ca2+ pulse, after 5-HT had been washed out, accelerated the rate of decay of cyclase activity to basal. These results suggest that the CS-US sequence requirement of classical conditioning may result from effects of Ca2+/calmodulin on Gs/cyclase coupling and uncoupling.

21.3 A MOLECULAR MECHANISM FOR MEMORY IN APLYSIA: UBIQUITIN-MEDITATED PROTEOLYSIS OF REGULATORY SUBUNITS OF THE CAMP-DEPENDENT PROTEIN KINASE. Ashok N. Hegde and James H. Schwartz. Howard Hughes Medical Institute, Columbia University, New York, NY 10032

In Aplysia, behavioral sensitization of defensive reflexes and the underlying presynaptic facilitation of sensory-to-motor neuron synapses lasts for several min (short-term) or days to weeks (long-term). Short-term facilitation has been explained by cAMP-dependent modulation of ion channel function. Long-term facilitation requires additional molecular processes including new protein synthesis. A key molecular event is persistent activation of the cAMP-dependent protein kinase (PKA). Gromen et al (Nature 326: 65-69, 1987) showed that regulatory (R) subunits are lost in long-term sensitization and this could account for persistent activation (Sweatt & Kandel, Nature 339: 51-54, 1989). Despite dependence on new protein synthesis, the loss of R results from proteolysis rather than diminished synthesis (Bergold et al, PNAS 87: 3788-3791, 1990). In order to understand the molecular events that produce the loss of R in long-term facilitation, we investigated how R is degraded. A prominent degradation pathway (involved in stress, cell cycle and other development events) is mediated by ubiquitin. When several molecules of ubiquitin are conjugated to a protein, it is degraded. We find that Aplysia R subunits in nervous tissue (and early embryos) are degraded through the ubiquitin pathway. First, high molecular weight Aplysia R-ubiquitin conjugates (ladders) form in cell-free rabbit reticulocyte lysates, suggesting that R subunits can be mult ubiquitinated. Second, we observe ATP- and ubiquitin- dependent degradation of R. We suggest that the ubiquitin-mediated proteolysis of R operates consistently in Aplysia neurons (which contain ubiquitin) and, further, that in facilitated sensory cells a protein targeting R for ubiquitination is discovered. The induction of this postulated targeting protein would result in enhanced degradation of R, which in turn would maintain PKA in an activated form.


Long-term presynaptic facilitation by 5-HT of sensorimotor synapses in culture is accompanied by increases in both the size of the EPSP evoked in the motor cell L7 and in a number of sensory neuron varicosities. By contrast, long-term presynaptic inhibition by FMRFamide of sensorimotor synapses is accompanied by a decrease in sensory neuron varicosities. Since in both cases the changes in the EPSPs are dependent on macromolecular synthesis, we now examined whether the structural changes also require protein or RNA synthesis. As previously reported, both transmitters evoked long-term changes in the EPSP in L7 that are paralleled by changes in the number of sensory neuron varicosities. Thus, the EPSP increased by 61% ± 10 and the number of varicosities by 46%/ ± 7 with 5-HT (N = 15), and the EPSP decreased -41% ± 4 and the varicosities decreased by -26% ± 5 with FMRFamide (N = 10). The protein synthesis inhibitor, anisomycin, and the RNA synthesis inhibitor, actinomycin-D, blocked both the changes in EPSP and in the number of varicosities evoked by each transmitter. The inhibitors alone had no significant effect on the structure of the sensory cell or the EPSP. These results indicate that structural changes require the expression of genes and proteins not required for the short-term changes, and suggest that the long-term structural changes are tightly coupled with long-term modulation in the efficacy of synaptic transmission.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991


We have found the synaptic growth that accompanies 5-HT induced long-term facilitation of the sensory to motor connection in Aplysia dissociated cell culture requires new protein and mRNA synthesis. This growth is affected by a down-regulation of adhesion molecules, by endocytosis, and by internalization of the L29-L30 neuronal complex. In current studies, we are using a novel method that allows us to block the process of cell surface rebinding of 5-HT induced adhesion molecules at the L30-L29 synapse. We have also found that L29-L30 interactions can serve as a module for the potentiation both directly (by L30 activation) and indirectly (by L29 activation).


We have developed an isolated mantle organ preparation for simultaneous behavioral and cellular studies of plasticity of the gill-withdrawal reflex in Aplysia. In our initial behavioral studies, we reinvestigated the effect on dishabitation and sensitization of test time, tap strength, and shock intensity. Sensitization occurred in most conditions, with significant dishabitation only under test conditions of 25 min after the shock, and the post-test was always slightly less than the first (post-pre-habituation test) 12.5 min after the shock. These effects of both tap and shock level (as in sensitization), and in most conditions the post-test was greater than the first test. This pattern of results suggests that at 25 min dishabitation of the tissue was less than removal of habituation, whereas at 12.5 min it may also be due to sensitization. In our initial cellular studies we reinvestigated the contribution of the LE mechanosensory neurons. We used a mechanical tapper driven by a solenoid and varied force by varying the travel distance. In 2 out of 21 preparations, a tap that did not actually touch the siphon elicited a gill-withdrawal response. By contrast, 32 out of 32 LE cells in those preparations fired action potentials if and only if the tapper touched the siphon. Moreover, the latency of firing of the LE neuron was always slightly longer than the latency of the response in a motor neuron. We have obtained similar latency results in a second series of experiments with a conditioned force stimulus. In these experiments, performs confirm that the LE cells participate in the reflex, and suggest that a second, unidentified population of sensory cells with lower thresholds and slightly shorter latencies may also contribute.


Activity-dependent facilitation of synaptic transmission from sensory neurons to motor neurons in Aplysia is thought to be due in part to enhanced production of CAMP in the sensory neurons (Kandel et al., 1983). Ocorr et al. (1988) more recently, Eliot et al. (1989) demonstrated activity-dependent facilitation in isolated cell culture, using a modified one-trial training procedure. We have begun to investigate whether this training procedure also produces electrophysiological changes consistent with enhanced production of CAMP in isolated clusters of pleural sensory neurons. In a first series of experiments, we produced tetanic forings of the neurons with a microelectrode shock either 0.5 sec. (paired) or 1 min (unpaired) before a 30-sec application of 5-HT and measured the excitability (number of spikes produced by a constant intra-cellular current pulse) of one cell in the cluster. Pairing produced a significantly greater index (K = 4.2 vs. 292%) and duration (K = K = 2.4 x 1.5 min) increase in excitability than unpaired training (K = 12 x 2.6, p < 0.5). Tetanus alone had no effect. Preliminary results from a second series of experiments suggest that pairing training also produces a greater increase in frequency-broadened spike width (12%) than either 5-HT alone (7%) or tetanus alone (-6%). These results are consistent with enhanced production of CAMP in the sensory neurons, and lay the groundwork for future experiments relating these electrophysiological changes to biochemical measurements in the same preparation.


Two forms of inhibition have recently been described in the siphon withdrawal reflex (SWR) of Aplysia: (1) a tail shock (TS) induces transient inhibition of the SWR (Marcus et al., 1988). This inhibition is mediated, at least in part, by inhibitory interneurons (INT). L16. L16 responds to TS, activation of which induces a reflex-induced contraction in the sensory neurons (MN), and voltage clamping L16 abolishes this TS-induced inhibition (Kandel et al., 1980, 1980). Inactivation of excitation INT L29 inhibits reflex-induced complex EPSPs in MNs; this inhibition arises in part from L29's recruitment of inhibition onto itself from inhibitory INT L30, and is modulated both by electrical coupling between the cells and by post-tetanic potentiation (PTP) of the L30-L29 inhibitory synapse (Fischer & Carew, 1989).

We have used the network simulator GENESIS to connect the connectivity and synaptic interactions described above into a biologically plausible model of the inhibition in the SWR. Our network consists of 2,102 simulated neurons, representing siphon and TS input, INT processing, and MN output. This network was trained with one form of inhibition (akin to the SWR: (1) Activation of the simulated L16 inhibits reflex-induced EPSPs onto MNs. Following TS, which activates L16, the EPSP is reduced by -75%. TS-induced inhibition is abolished under simulated voltage clamp of L16. (2) Activation of simulated L29 recruits inhibition onto itself via activation of INT L30, which in turn reduces excitation to MNs. We are currently examining the computational significance of the L29-L30 electrical connection and inhibitory PTP at the L30-L29 synapse.

As our model simulates inhibitory interactions in the SWR circuit, we will be interested to apply it to different forms of learning exhibited by this reflex.

521.10 LATERALIZATION OF LONG-TERM HABITUATION IN TAIL-INDUCED SIPHON WITHDRAWAL IN APlysia. M. Studer, X.V. Chan* and T.J. Carew. Department of Psychology, Yale University, New Haven, CT.

The tail-induced siphon withdrawal reflex (T-SWR) in Aplysia is mediated by bilateral clusters of sensory neurons. Each cluster encodes information from one side of the body, raising the possibility that learning mediated by these sensory neurons might be lateralized. Indeed, Scholtz and Byrne (1987) have demonstrated lateralization of long-term sensitization in the T-SWR. In this study we report that another form of learning, long-term habituation, also is lateralized in the T-SWR.

Aplysia were bilaterally implanted with silver wire electrodes so that weak electrical stimuli (behaviorally equivalent to tactile stimuli) could be delivered consistently to the same side of the animal. All experiments were conducted in freely-moving animals. Animals first received a series of bilateral pre-tests at a non-habituating (10 min) ISI; the T-SWR duration was measured following each stimulus. Next, computer-controlled unilateral habituation training ensued: four stimulus blocks were spaced 90 min apart (each block= 30 stimuli at a 10 sec ISI). Finally, bilateral interference tests were conducted. The results were not significant: in the first two experiments were conducted: in Exp 1 (N=13) the control side received no stimulus during the training period; in Exp 2 (N=15) the control side received an equivalent number of stimuli as the trained side, but at a non-habituating (10 min) ISI. An ANOVA revealed a significant overall effect of training in both experiments (interaction: p<0.01 in each). Furthermore, trained side test scores indicated significant habituation relative to their own pre-scores (Exp 1: p<0.05, Exp 2: p<0.001) whereas control sides showed no habituation.

Our results show that long-term habituation can be lateralized in the T-SWR. Since this reflex is well suited for a cellular and molecular analysis of behavior, it should now be possible to determine the extent to which our findings explore the detailed mechanisms of long-term habituation for this reflex.
DIFFERENTIAL EFFECTS OF THREE STIMULI PRODUCING LEARNED CHANGES OF RESPIRATORY PUMP RATE IN APLYSIA. M. Levy* and A. Saussez, Dept. of Life Sciences, Bar Ilan University, Ramat Gan 52900, Israel.

In Aplysia, the gill contracts during 2 behaviors: 1) the gill withdrawal reflex; 2) respiratory pumping. Learning affecting the withdrawal reflex has been extensively studied by others. We have examined learned changes in respiratory pumping.

Three stimuli affecting respiratory pumping were identified: 1) decrease in pH 2) increase and 3) decrease in salinity. Effects of all 3 have sharp thresholds. For any of the 3, threshold can be altered by pairing subthreshold stimulation (pH 7.0, 120% or 85% seawater) with head shock. Shock alone, pre-exposure to a subthreshold stimulus, or unpaired exposure to shock and a subthreshold stimulus lead to sensitization of respiratory pumping that is shorter-lasting than is effect of pairing. Thus, pairing may amplify sensitizing effects produced by shock and by the 3 stimuli.

The head ganglia and the abdominal ganglion contain neurons capable of sensitizing the gill withdrawal reflex. These neurons also affect respiratory pumping. When the pleural-abdominal connectives were cut, thereby separating the abdominal and head ganglia, no response was seen to decreased pH, but responses were normal to increases and decreases in salinity. These data suggest that sensitizing neurons in the head ganglia respond to decreased pH.

We tested this hypothesis further, by examining generalization of learning between the 3 stimuli. When animals were shocked in decreased pH and tested in either 85% or 120%, and vice versa, there was complete generalization of learning between the 3 stimuli. By contrast, there was no generalization of learning when animals were shocked in decreased pH and tested in either 85% or 120% seawater. These data support the suggestion that a common sensitizing site in the abdominal ganglion is responsive to increases and decreases in salinity, while a different site in the head ganglia responds to decreased pH.

DEVELOPMENT OF CEREBRAL CORTEX AND LIMBIC SYSTEM III
522.5 AN ANTISERUM TO THE LOW AFFINITY NERVE GROWTH FACTOR RECEPTOR LABELS SUBPLATE CELLS OF DEVELOPING NEOCORTEX: A CORRELATION OF SUBPLATE AND MARGINAL ZONE WITH THE SUBPLATE OF THE MOUSE AND A SUBPLATE OF GLIA IN TISSUE CULTURE. D.B. Wayne, A.A. Zupan, and A.L. Paulson Deps. of Cell Biology, Neurology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

The preplate cells of embryonic mammalian neocortex are a unique population of early-generated, predominantly transient cells that make up the subplate and marginal zone after cortical plate formation. They are closely associated with specific extracellular matrix components (Stewart and Pearlman, 1987; Chun and Sharz, 1988; Sheppard et al., 1990), and express the nerve growth factor receptor (NGFR) in the cat and ferret (Allendorf et al., 1990). Many preplate/subplate cells are neurons that form synapses and extend the first axonal axons (McConnell et al., 1985), but some may be glia (Rickmann and Wolff, 1985). To define this transient population further, we immunolabeled sections and dissociated cell cultures of the developing neocortex of the mouse with a new antiserum produced in the chicken to an extracellular domain of recombinant human NGFR (Zupan et al., Soc. Neurosci. Abs., 1991). The cell bodies and processes of subplate cells are clearly labeled by the antiserum on embryonic day (E) 16 and postnatal day 1. In cell cultures dissociated from cortex on E13 and double-immunolabeled three days later, the cell bodies and processes of a subset of the neurons identified with an antibody to neurofilaments (RM108 provided by Dr. V. Lee) are labeled with the NGFR antiserum, as are a subset of the glial cells identified with the monoclonal antibody R1/C (provided by Dr. M. Yamashita). Taken together, these findings indicate that subplate neurons express an NGFR-like molecule on their surface, and that NGFR expression also defines a subset of embryonic glial cells.

522.6 ANATOMIC AND FUNCTIONAL CORRELATES OF THE SUBPLATE IN THE SOMATOSENSORY CORTEX OF KITTENS. S.L. Juliano, R.A. Coda, D.E. Elin Department of Anatomy & Cell Biology, LSUHSC, Bethesda, MD 20814

In kittens less than 2 weeks of age, little stimulus-evoked functional activity (as revealed by 2-deoxyglucose [2DG] uptake) is seen in the somatosensory cortex. Dense metabolic label is present in these animals, however, as a band of increased activity at the interface between layer VI and the white matter. This region corresponds to the subplate, which is normally observed in developing animals. We found in kittens aged 3 days to 2 weeks, that the subplate was heavily labeled with 2DG uptake, whether or not a somatic stimulus was delivered. After this age, a metabolic correlate of the subplate was not visible and stimulus-evoked activity became gradually more apparent, until about 5 weeks of age, when it was adult-like. A dense band of cytochrome oxidase activity is also found in the same layer VI-WM location, normally not observed in the adult. Histologic evaluation of this region established an increased number of Nissl-stained cells at the layer VI-WM junction, which conformed in morphology to neurons of the subplate in addition, the cortical laminar organization of cell bodies is immature with layers V and VI being easily distinguished, while layers III-V are relatively indistinguishable. An immature pattern of acetylcholinesterase activity is also seen in animals less than 2 weeks of age. In the young kittens, a dense network of fibers extends from the WM to into layers V and VI. Cortical layers above this site possess only sparse AChE- fibers, in contrast to the adult distribution, which displays a dense distribution of fibers in all cortical layers. These structural correlates indicate that the subplate is present and metabolically active in the somatosensory cortex of kittens up to 2 weeks of age. Further studies may interpret the significance of the subplate in forming a mature pattern of functional activity in the somatosensory cortex. Supported by NS-24014.

522.7 MODIFICATIONS IN MYASTICAL VIBRISSE AND THE BARREL CORTEX IN RAGGED MUTANT MICE. J.C. Kuljis Department of Neurology, The University of Iowa College of Medicine, Iowa City, Iowa 52242-1053

The functional and anatomical alterations in the murine somatosensory cortex are among the most distinct examples of modular ensembles in the cerebral cortex. Postnatal cures from sinus hair receptors to this region are necessary for barrel development. However, since early prenatal vibrissal follicle ablation has so far been unsuccessful, virtually nothing is known about their role in the specification of barrels in embryos. The barrel cortex of 6 specimens of ragged, opposed (Ragged mutant mice and 4 controls was analyzed in coronal or tangential sections, using the Nissl, cytochrome oxidase and succinate dehydrogenase methods. Flat-mounted muzzle skin from each animal was then stained with von Kossa and Masson stains. The vibrissal pad of mutants has rows of small elevations or mounds in a location similar to that of sinus hair follicles and in a pattern identical to them in normal animals. Only 3-4 short and thin vibrissae originate from the most posterior of those mounds. Histology confirms that each mound - with and without vibrissa - is a sinus hair follicle. Barrels corresponding to the existing vibrissae are almost twice as wide as those not supplied by vibrissae, but exhibit about the same level of staining intensity for oxidative enzymes as the rest. Only barrels immediately surrounding the enlarged ones have a reduced diameter and a polygonal appearance, suggesting that they have either shrunk or grown less than their neighbors. These observations indicate that barrels can develop in the absence of pre- and postnatal vibrissal stimulation, but do not necessarily imply that they can develop in complete absence of cues other than action potentials from vibrissal receptors. It appears also that vibrissal stimulation may be a factor influencing the amount of cortex devoted to each barrel.

522.8 SPONTANEOUS ACTION POTENTIAL ACTIVITY AND SYNAPTIC CURRENTS IN THE EMBRYONIC TURTLE CEREBRAL CORTEX. Mark G. Blanton and Arnold R. Kriegstein, Department of Neuroscience and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305

We used loose-patch and whole-cell recording techniques to study spontaneous action potential activity and excitatory and inhibitory synaptic currents in embryonic neurons in the cerebral cortex of mice. Spontaneous action potentials appeared early in development at stage 17. As the cortical plate matured in mid-embryonic stages, action potential activity became more regular and fell into one of two distinct patterns, short waveform, basically tonic neurons (pyramidal) and fast waveform, repetitively firing neurons (nonpyramidal). Spontaneous synaptic currents with rapid rise and slow exponential decay were observed in embryonic neurons at stage 18. Spontaneous excitatory synaptic currents appeared first, and while IPSCs could be elicited by repetitive stimulation at this stage, spontaneous IPSCs appeared suddenly at stage 20 and were surpassed by EPSCs in frequency. These results, and the presence of inhibitory synapses ultrastructurally at stage 18 (Pamavelas, Blanton and Kriegstein, unpublished), indicate that the time of onset of spontaneous IPSCs is not determined by the formation of a functional synaptic apparatus but is dependent on a later developmental event. EPSCs increased in frequency, rise time, and tau, but amplitude remained stable despite dendritic elongation. In contrast, IPSCs showed a significant change in mean amplitude, increasing from 9.0 ± 0.6 pA at stage 18 to 13.0 ± 2.2 at stage 26 at a holding potential of -20 mV, and showed no change in tau. IPSCs and EPSCs were sensitive to TTX, and were thus dependent on spontaneous action potential activity. Spontaneous IPSCs appear just prior to the time excitatory synapses can synchronize epileptiform bursts, and after the time inhibitory activity can be evoked. Inhibitory synapses are therefore present in advance of their role in controlling excitability and are recruited as excitatory activity increases. Spontaneous neuronal activity emerges in a characteristic sequence and provides a substrate for shaping development in vivo.

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MOTORNEURON IN model system for studying the role of neuromodulation and co-transmission. These peptides fail to modulate ARC contractions when applied exogenously. These peptides fail to provided indirect evidence for SCP release in the ARC. We have developed a method for directly measuring SCP and buccalin release and provide indirect evidence for SCP release in the ARC. The results provide good evidence that the peptides are released in vivo when the animal feeds.

523.3 Rfα PEPTIDES ARE PRESENT IN THE ARC NEUROMUSCULAR SYSTEM OF APTYSIA. L. Cropper, F. Villan, V. Weiss, M.W. Miller, K. Weiss. Dept. Physiol. & Biophys., Mt. Sinai Sch. of Med., NY NY 10029; Cntr. Neurobiol. & Behavior, N.Y.N. State Psychiatric Inst., New York, NY 10029. A striking feature of the arcu formula system is its chemical complexity. The ARC motor neurons B15 and B16 each contain at least four neuropeptides in addition to their primary neurotransmitter ACh. All four of the B15 peptides appear to be packaged in the same dense core vesicles, and therefore are presumably coreleased. Thus, modulatory effects of the ARC motor neurons B15 and B16 are likely to be exerted independently. When peptide cotransmission occurs, it is likely that multiple peptides are released, and that released peptides have interactive effects. This suggests that a comprehensive investigation of peptide cotransmission in the ARC motor system will be difficult unless all major bioactive peptides present in B15 and B16 are characterized. Towards this end, material was extracted from neuronal processes attached to 100 g of ARC muscle and was subjected to preparative chromatography. The active fractions were pooled and the following amino acid sequences were obtained: G-A-L-R-F-R-F, G-S-L-F-R-F, and S-T-L-R-F-R. To localize these peptides, and to verify their structures, B15 neurons were incubated in 3H-Phe and chromatographic properties of radiolabeled peptides were compared to those of synthetic peptides. The precursor sequence includes two novel related peptides, SCP and myomodulin (MM) are contained in the ARC muscle. Probing Northern blots of the precursor sequence includes two novel related peptides, SCP and myomodulin (MM) are contained in the ARC muscle. Probing Northern blots of the precursor sequence indicates that a comprehensive investigation of peptide cotransmission in the ARC motor neurons B15 and B16 and increase ARC contraction amplitude and relaxation rate. SCP is known to increase ARC CAMP levels, the MM second messenger is unknown, but is not CAMP. Application of p-32-PATP or forskolin, an adenyl cyclase activator, resulted in dramatic increases in ARC contraction amplitude and relaxation rate; threshold concentrations for each was 10^-3 M. SCP application and B16 stimulation, but not MM application or B16 stimulation alone increased CAMP levels. This enhancement of CAMP by the modulators may underlie their potentiation actions. This enhancement of CAMP by the modulators may underlie their potentiation actions. This enhancement of CAMP by the modulators may underlie their potentiation actions. This enhancement of CAMP by the modulators may underlie their potentiation actions.
523.7

FURTHER CHARACTERIZATION OF NEUROPEPTIDES THAT
MIMIC PROLONGED INHIBITION OF BAG CELL TARGET
NEURONS IN APLYSIA. S.M. Rajpara* , J.C. Eliassen* and E.
Mayeri. Department of Physiology, University of California, San
Francisco, CA 94143

The bag cell neurons of Aplysia are part of a neural system that
utilizes four peptides as neurotransmitters. The peptides are
derived from the egg-laying hormone/bag cell peptide (ELH/BCP)
precursor and produce various effects in abdominal ganglion
neurons following injection of a bag cell burst discharge. In cells
L3 and L6, bag cell activity produces inhibition that lasts for 2 or
more hours. a-BCP mediates an early component of this
prolonged inhibition.

To further characterize two peptides that are candidates for
mediating the prolonged component of inhibition, we purified material
from an acid extract of abdominal ganglia by molecular
sizing HPLC, followed by one or more steps of reverse-phase
HPLC on C4. One peptide is 4878 Da and consists of 40 amino
acids, as determined by mass spectroscopy and amino acid
composition analysis. A two min. application of the peptide at a
concentration of 4 μM produced sustained hyperpolarization and a
reduction in spontaneous spike activity that lasted 100 min. The
second peptide has an apparent molecular weight of 3300 Da and
was 5-fold less potent. Amino acid composition analysis suggests
that neither peptide is derived from the ELH/BCP precursor.

Since the 4878 Da peptide is effective at a lower concentration, it is
the best candidate for the true inhibitory transmitter.

Supported by NIH grants NS 16490 and NS 16033.

523.9

SEROTONIN AND FMRFamide INHIBIT THE
RELEASE OF NEUROPEPTIDES FROM IDENTIFIED
APLYSIA NEURONS IN CULTURE. M.D. Whim and P.E.
Chicago, Chicago, IL 60637.

To examine the modulation of the release of neuropeptide
cotransmitters we have used Aplysia neurons B1 and B2 in primary
culture which incorporate 35S-met into 2 neuropeptides (SCPs and
 SCPs). The SCPs are transported into the regenerated neurites, and
can be shown to be released in a stimulus- and Ca-dependent fashion
(Lloyd et al. 1986). Initial experiments determined that stimulating
individual B1 or B2s using intracellular microelectrodes at a
frequency of 6 Hz for 500 msec with 500 msec interburst intervals,
evoked a reproducible release of radiolabeled SCPs into the bathing
solution which could be detected using a combination of HPLC and
liquid scintillation counting. This release showed only a small decline
with repetitive stimulation periods. Bath application of FMRFamide
and serotonin both transiently inhibited release. 4 μM FMRFamide
reduced the release of the SCPs to between 5% and 15% of control.
Inhibition by serotonin was dose-responsive, with a ~60% inhibition of release
at 100 μM. Although serotonin inhibited the release of the SCPs, it
increased excitability as monitored by the number of spikes evoked
by depolarizing current pulses. We are currently examining the
possibility that the effects of serotonin are mediated via the actions of a
second messenger. Supported by NS 23569.

523.11

DISTRIBUTION OF THE NEUROPEPTIDE APGW-NH2 IN THE
CENTRAL NERVOUS SYSTEM AND MALE REPRODUCTIVE
ORGANS OF LYMNAEA STAGNALIS, J. van M Innin, A.B. Smit*
and P.P. Croll. Faculty of Biology, Vrije Universiteit, 1081 HV
Amsterdam, The Netherlands and 1Department of Physiology &
Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H
4J7.

Recently, a gene has been cloned from neurons located in the anterior
lobes of the cerebral ganglia of Lymnaea stagnalis. The product of this gene
is processed to become 10 copies of the neuropeptide APGW-NH2 and
a single copy of a 40 amino-acid polypeptide. The present study
describes the distribution of neurons expressing the APGW-NH2 gene by means of
in situ hybridization and immunocytochemistry in the CNS and
male reproductive organs. The majority of the neurons expressing
APGW-NH2 is located in the anterior lobes of the cerebral ganglia.
The right lobe, which contains largely of motoneurons innervating the Buccal
muscle I3a is innervated by two excitatory motor
neurons, B3 and B38 and a newly identified inhibitory neuron,
B47. B3, B38, and B47 synthesized FMRFamide, the SCPs,
and myomodulin (MM) respectively. Bath application of
FMRFamide, SCPs or Mm (10 nM) enhanced B3-evoked
contractions. We monitored the modulation of B3-evoked
muscle contractions in response to stimulation of B3 and B47
using paradigms designed to release conventional transmitters
alone and with peptide cotransmitters. When B38 was stimulated
in high frequency bursts during B3 interburst intervals,
subsequent B3-evoked contractions were enhanced. We interpret
these results as B38 releasing its peptide modulatory
cotransmitters (SCPs) during high frequency bursts. Stimulation of
B47 simultaneous with B3 inhibited the B3-evoked
contractions. However, high frequency B47 stimulation during
B3 interburst intervals enhanced subsequent B3-evoked
contractions. It appears that B47 can have different effects on
neurally evoked muscle contractions depending on the
temporal characteristics of stimulation. We interpret these results
as B47 releasing its inhibitory conventional transmitter alone
during low frequency bursts and along with its peptide
cotransmitter (MMn) during high frequency bursts. Supported by
NS 23569.

523.8

MODULATION OF NEUROMUSCULAR EFFICACY
BY EXCITATORY AND INHIBITORY MOTOR
NEURONS IN APLYSIA. P.J. Church and P.E. Lloyd.
Committee on Neurobiology, The University of Chicago, Chicago, IL 60637.

By studying the peripheral expression of the FMRFamide gene
sequence in Aplysia, we have identified a critical role for the
protective peptide in the modulation of the neuromuscular
transmission at the level of the muscle fiber. Synthesis and
release of FMRFamide in a stimulated neuron results in
modulation of the excitability and motoneuron activation pattern.

Supported by NIH grants NS 16490 and NS 16033.
524.1

THE ROLE OF HORMONES IN REGULATING THE MITOTIC ACTIVITY OF NEUROBLASTS IN THE MOTH, MANUDA SEXTA. B. Booker and J. Kim

Section of Neurochemistry, The University of New York, NY 10027.

The neurons generated by postembryonic neuroblasts (NBs) play an important role in shaping the nervous system of the adult Manduca. Each NB generates a discrete nest of progeny. The NBs are individually identifiable, and each NB generates a characteristic number of progeny, ranging from 10 to more than 100. We examined mitotic activity of the NBs, using immunocytochemical detection of the thymidine analog 5-bromo-2-deoxyuridine, in combination with a series of endocrine manipulations.

The mitotic activity of the NBs was not uniform throughout the larval stage. There was little change in the mitotic activity of the NBs throughout the feeding larval stage. However, at the onset of metamorphosis, which occurs on the first day of wandering, the mitotic activity of the NBs increases approximately 20-fold, peaking at 5-3. The mitotic activity of the NBs dropped slowly over a period of 10 days, parallel to the appearance of the large prepupal peak of ecdysteroids. Ligating abdomens on W-0 prevented this increase in mitotic activity. Replacing the ecdysteroids with an infusion of 20-hydroxyecdysone (20-H) dramatically increased the mitotic activity of the NBs. Infusion of 20-H into animals treated for 14 days with JH, which prevents the cessation of mitotic activity triggered by the small peak of ecdysteroids, did not increase mitotic activity. This suggests that the number of progeny a NB generates is determined by intrinsic cues. However, our endocrine manipulations suggest that hormone activity can modulate the mitotic activity of the NBs at the onset of metamorphosis development.

524.2

CLONAL ANALYSIS SHOWS NOTCH IS REQUIRED FOR NORMAL SENSILLAR DEVELOPMENT IN THE WING OF DROSOPHILA. E. Ridolfi, N. Stuart, and T. B. Blatt.


The techniques of mitotic recombination and enhancer-trap based cell marking were used to generate mosaic clones of a null allele of the Notch gene in the wing of Drosophila. It is thought that cell-cell interactions mediated by Notch protein is a receptor for a lateral inhibitory signal; this signal is required to produce the normal number of correctly positioned sensilla within a field of corpora. Preliminary results showed that mutant clones of Notch in the wing produced supernumerary sensillum precursors surrounding the positions of normal calyciform sensilla. However, clones of mutant cells were seen to contain clusters of supernumerary neurons. Thus, in the wing, as in the notum, Notch may be required by competent cells for the generation of the correct pattern of sensilla.

524.3

ANALYSIS OF A DROSOPHILA GENE CLUSTER EXPRESSED DURING CNS DEVELOPMENT. S. D. Zhang, D. Mellier-Dressler, H. Gainer, W. Odenthal, and J. Kassell.

Lab. of Neurochemistry, NINDS, NIH and Ctr for Biologics Evaluation and Research, FDA, Bethesda, MD 20892.

Enhancer-detection screening has led to the identification of a gene cluster located at 83c which is active during Drosophila neurogenesis. Thus far, two non-overlapping genes (83c alpha and beta) that are transcribed in converging directions have been partially characterized.

Genomic and cDNA analysis indicated that 83c alpha may encode a transcriptor factor. The deduced protein sequence contains an acidic domain and four linked TFIIA-like zinc fingers. Whole mount in situ hybridizations revealed that 83c alpha gene was first activated at the time of neuroblast formation in clusters of cells within the cephalic neurogenic region. Simultaneously, the 83c alpha expression is observed in groups of three to four cells located in the ventral cord midline. Each group is positioned at the anterior portion of each parasegment. During ventral cord development, 83c alpha gene displays a progressive anterior to posterior bilaterally symmetrical waves of activation in cells flanking the ventral midline. These cells may be neuroblasts. Late in CNS development a cluster of 83c alpha gene expression is observed in both the cephalic lobes and in the ventral cord. Except for four to six cells along the posterior boundary of the contracted ventral cord, no transcripts are detected in the abdominal ventral cord.

Sequence analysis of genomic and cDNA 83c beta clones indicates an open reading frame of at least 2kb. The encoded protein bears homologies to previously described proteins. 83c beta transcripts are detected in early embryos, suggesting this gene is maternally expressed. Late in embryonic development 83c beta is expressed throughout the CNS. However, unlike 83c alpha, expression of 83c beta is not limited to the CNS.

524.5

CRITICAL PERIOD OF INTERACTIONS LEADING TO THE BIRTH OF PERIPHERALLY INDUCED CENTRAL (PIC) NEURONS DURING LATE EMBRYOGENESIS IN THE LEech, HIRUDO MEDICALIS.

Thomas Hecker and Eduard R. Macagno. Dep. of Biological Sciences, Fairchild Center, Columbia University, NY, NY 10027.

PIC neurons are present only in the sex ganglia (those in the fifth and sixth body segments) of the midline. They appear as a result of an inductive interaction between the male genitalia and the CNS (Baptista et al., Nature 346, 855-858; 1990). This interaction requires the presence of the male genitalia and the sex ganglia. Male organ removal prior to embryonic day 13 (E13) prevents the appearance of PIC's entirely. Conversely, at E16, the removal of the peripheral target no longer prevents the birth of PIC's (Baptista and Macagno, J. Neurobiol., 19, 707-726; 1988). To explore the basis of this critical period of PIC induction, we performed a series of male organ ablations at E10-11, followed by back-transplantation of male organs at later stages from donors of the same age. PIC neurons only appeared in animals that received a back-transplant up to E16. To test whether this resulted from the loss of male organ after E11 or was opposed to a loss of CNS responsiveness, we performed heterochronic transplantations. Organs from animals up to E40 were able to induce PIC's in E13 blastomeres. Animals that had lost this ability by E10 no longer responded to PIC inducer. We conclude (1) that the interaction can take place in as short a time as one day and (2) that the sex ganglia lose their sensitivity to the inductive signal after day 16, although the male organ retains its ability to induce up to E 40.

524.4

POSTEMBRYONIC NEUROGENESIS AND DEVELOPMENT OF THE ANTENNAL LOBES IN THE TOBACCO HORNWORM MANUDA SEXTA. K.A. Strowick, N.T. Davis, and J.O. Hildebrand. ARK Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Manuda sexta exhibits dramatic changes in its appearance and behavior during its metamorphosis from larva to adult. These changes are accompanied by extensive reorganization of the nervous system, involving the death and remodelling of subsets of larval neurons as well as the postembryonic addition of new neurons. We are studying the origins of neurons in the primary olfactory center, the antennal lobe, in the brain of M. sexta. We previously identified 5 neuroblast nests (3 lateral, A-C, and 2 medial, D,E) that, based on their positions adjacent to the larval antennal center, appeared likely to contribute to the reorganization of this structure into the adult antennal lobe. We are now interested in determining how these 5 nests relate to the 3 groups of neurons (lateral, medial, and anterior) found in the adult antennal lobe. To determine the contributions of these five nests to the development of the adult antennal lobe, successive stages of prepupal and pupal development were examined in histological and histochemical preparations. Cell death is evident within the nests from stage W0-W2; nest-cell processes appear as prominent tracts leading from each nest to olfactory neuropil from stage W3 forward. The olfactory neuropil remains larval in character (each nest having its own discrete tract and characteristic point of entry into the olfactory neuropil) until stage P2, after which somata and tracts associated with each of the lateral nests (A-C) appear to coalesce, forming a characteristic adult lateral cell group and input tract by stage P4. Medial nest retains their orientation and input tracts relative to the neuropil during metamorphosis, with nest E appearing to contribute to the medial cell group of the adult antennal lobe and nest D, to the anterior cell group. [Supported by NIH grant AI-22531.]

524.6

REGULATION OF NEURONAL NUMBER AND SIZE IN FROGS DEVELOPING FROM HALF EMBRYOS. Francesca Marta and Margaret Halloway.

Department of Biology, Bryn Mawr College, Bryn Mawr PA 19010.

The developmental processes which determine neuron number and its relation to body size in Xenopus are not well understood. To explore this issue, we studied embryos developing from fertilized eggs of Xenopus laevis which were divided at the two-cell stage. Single blastomeres were isolated using micromanipulation techniques. Five treatments were used: experimental embryos and their matched controls to compare cleavage patterns, rate of development and growth. Embryos were raised as whole mounts with the lowered axis of the explant. When the time of axial displacement was reached, they were processed for light and electron microscopic analysis. Quantitative estimates of body size were obtained from stained serial sections using a camera lucida and digitizing software. Counts were made of regions selected to estimate total neuron number. The oldest embryos studied to date were from stage 48-49 of Nieuwkoop and Faber.

Experimental embryos showed altered early cleavage patterns; the isolated blastomere displayed an additional, vertically oriented cleavage division before cleavage horizontally. Nevertheless, both experimental and control embryos underwent neurulation at the same time, despite the diminished size of the experimental embryo. The experimental embryos were consistently smaller than their matched controls throughout the observation period. Analysis of body size and total neuronal number in the tadpoles revealed that experimental embryos were approximately half-sized compared to control embryos and that their nervous systems contained half the number of neurons as did controls. These preliminary results suggest that both body size and neuronal cell number are heavily influenced by the volume of cytoplasm in the egg.

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Environmental cues are known to influence the differentiation of neural crest cells and could also influence their genesis. We examined neurogenesis in a gnotobiotic chicken strain in which we have been able to maintain a prolonged period of development. The cardiac ganglion of the frog Xenopus laevis contained a single type of neuron whose precursors continued to divide and differentiate long after neural crest cells had been depleted. We located a time dependence of the neural crest precursors and determined that their proliferative ability differed from precursors that had just completed migration from the neural crest.

A NOVEL MARKER FOR CORTICAL SUBPLATE NEURONS AND SOME SUBVENTRICULAR ZONE CELLS IN EARLY POSTNATAL RAT BRAIN. Z.Y. Tu and J.E. Bottenstein. Marine Biomedical Institute, University of Texas Medical Branch in Galveston, Galveston, TX 77550

Our previous work has shown that conditioned medium (CM) from the B104 CNS neural cell line increases proliferation of O-2A glial progenitors and immature oligodendrocytes. We generated mouse monoclonal antibodies (mAbs) against a partially purified heparin-binding fraction of the CM from this cell line. One of these antibodies (AC3) was used in combination with mouse mAbs against G0/Ki67 antigen (a nuclear marker) or against G3a ganglioside (a marker for immature neuroepithelial cells) to identify the cells expressing the AC3 antigen. A double immunoperoxidase method was used on 60 μm thick sections of early postnatal rat brain. Strong AC3 immunoreactivity was observed within cells of the cortical subplate in P0 and P7 (postnatal day) rat forebrain, but it disappeared by P14. The majority of these AC3-positive cells double stained with several MNF mAbs, indicating that they were neurons. In the subventricular zone (SVZ), especially in the medial portion between the lateral ventricle, strong AC3 immunoreactivity was detected in cells double stained with an early neuroepithelial marker (B1). An apparent decrease in AC3-staining is observed in the subventricular zone of the cortex compared to the subplate. An apparent decrease in AC3-positive SVZ cells was seen at P7 and P14. Only a few AC3-positive cells in this region were double stained by G3a mAbs. However, strongly G3a-immunoreactive cells were observed in the cingulum, corpus callosum, and other parts of the SVZ. No double staining was detected for AC3 and G3a in the cingulum, corpus callosum, or cortical subplate. Our results indicate that AC3 mAbs provide a novel marker for cortical subplate neurons and for some SVZ cells and may be useful in studies of the maturation and migration of cells in rat brain.


Recent studies have demonstrated that a subset of nuclear proteins which bind DNA are involved in the regulation of gene expression during development. It is our long-range goal to characterize the expression of these proteins in vitro. Using both affinity chromatography to isolate DNA-binding proteins and hybridoma technology, we are producing monoclonal antibodies against nuclear proteins in the nervous system of the chick. We report here on 4 MAbs which were made against mouse brain nuclear protein (A00), A18, F41, Smith et al., Soc.Neurosci.Abul. 16: 642, 1990), which recognizes the nuclear matrix, and two new MAbs (1Vp and 2Vd) which stain chick neuronal cell nuclei. The new MAbs were produced by immunizing mice with protein isolated from chick brain nuclei. Total soluble nuclear protein was loaded onto a DNA-cellulose column and eluted with high molar salt. Silver stained gels of eluted fractions showed that most proteins were in the flow-through fraction, but that high salt elutions contained proteins predominantly in the 35-70 kDa range. Immunoblot analysis has been performed for these four antibodies in the chick and will be presented.

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BIRTH AND MIGRATION OF CELLS IN THE CENTRAL POSTERIOR NUCLEUS OF WEAKLY ELECTRIC KNIFEFISH (EUGENANNA SP.), DURING ADULTHOOD. H. M. Zupanc, J. M. Zupanc and M. M. Zupanc. California Academy of Sciences, Institution of Oceanography, 1Department of Neurosciences, and Department of Biology, University of California at San Diego, La Jolla, CA 92037.

In Eugenanana sp., a weakly electric fish, a bilateral, prosencephalic, mesencephalic cell group, stretching from the third ventricle approximately 400 μm laterally. At its lateral aspect, the CP fuses with the preoptic nucleus (PPN), which controls frequency modulation of the electric organ. The histochemical and morphological data have suggested that the CP is much larger than assumed in earlier studies (e.g., M. Kawasaki et al., J. Comp. Neurol. 276:113-131, 1988) and that it incorporates at least part of the traditionally defined CP (G.K.H, Zupanc and W. Heiligenberg, submitted). Furthermore, experiments employing 5-bromo-2-deoxyuridine (a thymidine analogue which is incorporated in replicating DNA and which enables the detection of mitotic S-phase cells) in adult knifefish revealed the generation of approximately 20-50 new cells per 12 hrs unilaterally in the periventricular zone of the CP (G. K. H. Zupanc, Soc. Neurosci. Abstr. 16:128, 1990).

In the present autoradiographic study, we examine the fate of these newborn cells. Fish were injected with 5-bromo-2-deoxyuridine and sacrificed after various lengths of survival time. While 5-bromo-2-thymidine labeling was highly concentrated in the periventricular zone after 12 hrs (mean, median distance of labeled cells from ventricle = 8 μm; n = 135 cells examined), newborn cells appeared to migrate laterally on the following days (day 2: m = 17 μm, n = 106; day 4: m = 27 μm, n = 41). The median distance of labeled cells from the ventricle reached a plateau after about one week (day 7: m = 29 μm, n = 177; day 7: m = 29, n = 111; day 28: m = 29 μm, n = 38). Light and electron microscopic observations suggest that at least part of the newly generated elongated periventricular cells differentiate into round or ovoidal neurons of the CP during their lateral migration.


The development of the mammalian cerebellum involves a complex system of cell-cell interactions which are controlled by both intrinsic genetic programs and epigenetic factors. To define the molecular events underlying cerebellar development, we have initiated a search for genes which mark specific stages in the generation and (GC) differentiation. Polyclonal antiserum made in rabbits against purified postnatal days (P) 3-5 GC was adsorbed on PC12 cells and used to screen a cDNA library made in AG11 from purified P3-5S GC (Ross et al., Soc. Neurosci. Abst., Vol.16 PI p.151, 1990). Of 58 purified phage clones, 42 were found to be unique by restriction enzyme analysis, but they were not amplified. Search of all GenBank entries with 100-500 nucleotides of 5' sequence showed 36 novel clones. Northern blots of poly A+ RNA made from cerebella of mouse embryos up to P17 showed a variety of mRNAs. A number of mRNAs have shown that six of the 26 clones analyzed thus far are developmentally regulated. Five of these six are brain-specific. In situ hybridization has shown localized expression of several of these subclones to cellular subpopulations in the mouse P8 cerebellum. For example, clones whose expression is restricted to superficial layers of the EGL, or to the outermost cells of the IGL have been identified. Our present efforts are directed toward identifying which of these novel genes require specific cell-cell interactions for expression, and toward understanding their role in cerebellar development.

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The 5A1 antigen is a unique adhesion molecule identified in the neural retina that participates in neuroepithelial interactions. Three antibody probes have been prepared to this antigen to facilitate its characterization during retina development. Immunohistochemical analysis shows that the antigen is ubiquitous on retina cell plasma membranes early in development but later becomes restricted to Muller glial cells, photoreceptor cells and the pigmented epithelium. By Western blot analysis, all antibodies identify a doublet at 45-45kDa with and without reduction. An additional band at 68kDa is observed in retina only. Lectin and endoglycosidase analyses of the antigen indicate that it is an N-linked 45kDa glycoprotein. Immunohistochemical analysis shows that the antigen is recognized by the probes. Lectin and endoglycosidase analyses of the immunopurified antigen revealed similar characteristics of the glycoprotein antigen: the antigen contains a single band of 45kDa in electrophoresis. The 45kDa band seems to be distinct from the others in its glycosylation and peptide core. Microsequencing of immunopurified 45kDa material has produced sequences that show little homology with sequences in the available data bases. PCR was used to produce and then clone additional sequence from retina mRNA. Again the sequence appears to be unique. Supported by NSF grant # BNS-8817943 to PJL.
Delayed Phosphorylation Of GAP-43 During Neurite Outgrowth in a Mouse Neuroblastoma Line

525.3 NEURON-SPECIFIC PROMOTER CONSTRUCTS FOR OVEREXPRESSION OF B-50(GAP43) IN TRANSGENIC MICE.

525.4 APPROACH TO STUDY GAP-43 FUNCTION BY HOMOLOGOUS RECOMBINATION-MEDIATED GENE TARGETING IN EMBRYONIC STEM CELLS.

525.5 LOCAL REGULATION OF GAP-43 PHOSPHORYLATION IN GROWTH CONES OF NEURITES REGENERATING IN CULTURE.
525.7 ALTERNED ORGANIZATION OF INTERMEDIATE FILAMENT PROTEINS IN GAP-43 DEFICIENT PC12(B) CELLS. J.P. Hammond, K.K. Meti and E.E. Basche. 1Cell and Molecular Neurobiology, Brooklyn-Queens Squibb College, Wallingford, CT 06492. 2Department of Pharmacology, Squibb Health Sciences Center, Syracuse, N.Y. 13210. Intermediate filaments are tissue specific cytoskeletal proteins that influence cell shape and function. To determine whether the growth-associated factor (GAF) 1 is involved in intermediate filament organization in PC12 cells, we compared the pattern of intermediate filament expression in GAP-43 deficient PC12(b) cells (Basche et al. p. 210-215, 1991) with line 15, a "GAP-43 deficient" line. Furthermore, GAP-43 expression in the PC12(b) cells was restored by transfecting the cells with vectors containing the GAP-43 cDNA together with the genes for the hypomyosin B or neomycin resistance. Cells were transfected with NGF to induce neurite formation for 3-7 days. All cells were processed for immunofluorescence using antibodies to the neurofilament proteins and to peripherin. GAP-43 containing and GAP-43 deficient PC12(b) cells exhibit neurofilament immunoreactivity within the cell body and extending out to the end of the neurites. The distribution of peripherin was markedly different. In PC12(b) cells peripherin immunoreactivity was confined to the cell body and was absent in the neurite processes, whereas in "GAP-43 normal" PC12 cells, or in the GAP-43 transfected PC12(b) cells, the peripherin was distributed within the neurites. The GAP-43 deficient PC12 cells will be useful for the investigation of the role(s) of GAP-43 in neuronal development and function.

525.9 POST-TRANSINCATIONAL MECANISMS DURING PROCESS OUTGROWTH II. Stabilization of GAP-43 and other neuronal mRNAs in TPA induced PC12 cells. J.H. Folstad, N.T. Kish and N.I. Perrone-Bizzozero. Department of Biochemistry, University of New Mexico, Albuquerque, NM, 87131-5221, U.S.A. Previous work from our laboratory showed that the expression of the neuronal growth-associated protein GAP-43 is induced by nerve growth factor (NGF) via a selective change in the rate of degradation of the mRNA (Perrone-Bizzozero et al., 1990). Neuronal, Abs. 10, 814. To study this further, we have studied the effects of NGF and other agents that modulate neurite outgrowth on the steady-state levels and on the relative rates of decay of GAP-43 and other neuronal mRNAs. By Northern blot analysis, we have found that GAP-43 mRNA levels in PC12 cells were increased in response to the phorbol ester TPA, but not by either dibutyryl-cAMP or the Ca+ ionophore A23187, suggesting that GAP-43 expression in PC12 cells depends on the activation of PKC. Furthermore, TPA had a much greater effect than NGF on both the onset and extent of process outgrowth and on the levels of the mRNAs for the neuronal proteins GAP-43, β-tubulin II (β-tub) and actin. In mRNA decay studies, TPA was found to increase 3-4 fold the half-lives of GAP-43, β-tub and actin mRNAs but had no effect on that of the house-keeping enzymes deoxyribonucleoprotein (C3PD). The effect of TPA on the stability of GAP-43 and β-tub mRNAs was blocked by dexamethasone but not by cycloheximide, suggesting that post-translational modification of a neuronal factor is required for these effects. In conclusion, our results indicate that in addition to transcriptional control, post-translational mechanisms may play an important role in the regulation of gene expression during neuronal outgrowth. Supported by NSF (NSF-R011199) and the American Paralysis Association (PBI-9006) to N.P.B.

525.11 FUNCTIONAL CONSERVATION OF GAP-43 GENE REGULATORY ELEMENTS BETWEEN MAMMALS AND FISH. E. Reinhard*, E. Nedivi. J. Neurosci. Soc. 125, 201-208. Each of the best characterized examples of a gene that exhibits both neural-specific induction and later axon-dependent repression in neurons is the single-copy gene encoding GAP-43. In order to analyze regulatory elements controlling expression of this gene, we have now isolated a functional GAP-43 promoter from rat, and 5 kb of DNA flanking this promoter. A 386 bp fragment containing the GAP-43 promoter is fused to a neomycin resistance gene and is introduced into PC12 cells, whereas the 5 kb fragment was activated also in more posterior regions of the nervous system. Our results demonstrate that this paradigm can be used to study neurite plasticity and the regulation of GAP-43 in vivo. Supported by LEAD 40616, ROI AG 0112, and NIH NS25778.

525.8 GAP-43 MESSAC LEVELS IN A HUMAN NEUROBLASTOMA CELL LINE BEFORE AND AFTER DITERMINATION BY RETINOE ACID. W. Neumann*, J.F. Tolksdorf*, E. Rogers, J. Sullivan, M.D.E. Notter, P.D. Coleman, Dept. Neurobio. and Anes, Univ. of Rochester Rochester N.Y. 14642. It has been shown that neuroblastoma cells can be differentiated in vitro to acquire phenotypes that are absent prior to differentiation. These include the expression of neuronal surface markers, electrical properties, cessation of cell division, and induction of morphological characteristics, including the extension of neurites with growth cone formation. The ability of this system to represent a model of destruction of neurites is 50 fold increase in GAP-43 message levels as compared with controls, normalized for oligo dt blot hybridization. Northern blot analyses ensured intact RNA and confirmed the identity of the GAP probe, which was made by random priming of a full length GAP-43 cDNA. It is suggested that this paradigm can be used to study neurite plasticity and the regulation of GAP-43 in vivo. Supported by grants HD2448 and EY03797.

525.10 IDENTIFICATION OF cis-Acting ELEMENTS REGULATING GAP-43 PROMOTER ACTIVITY. E. Nedivi*, G. S. Bai, and J. H.P. Skene. Dept. of Neurobiology, Stanford University, Stanford, CA 94305. We have isolated a functional GAP-43 promoter from rat brain and characterized its function in PC12 cells. This promoter contains approximately 270 bp of 5' DNA flanking this GAP-43 gene. Since the GAP-43 gene is only expressed in neurons, we hypothesize that this region contains cis-acting elements responsible for early neural-specific expression of this gene. Our results indicate that in addition to transcriptional control, post-translational mechanisms may play an important role in the regulation of gene expression during neuronal outgrowth. Supported by NSF (NSF-R011199) and the American Paralysis Association (PBI-9006) to N.P.B.

525.12 GAP-43 mRNA BINDING PROTEINS RELATED TO NEURAL DIFFERENTIATION. N. Irwin, N. I. Perrone-Bizzozero, and J. I. Bennetow. Dept. Neurosurgery, Children's Hospital, Harvard Med. School, Boston MA 02115; Dept. Biochem., U. New Mexico. Expression of the membrane phosphoprotein GAP-43 is closely linked to the development and reorganization of neuronal connections1. Although growth-related changes in GAP-43 are generally paralleled by changes in levels of the mRNA, nuclear run-on studies indicate that these mRNA changes do not reflect altered rates of gene transcription. For a number of other mRNAs, regulation involves the binding of cytoplasmic proteins to defined nucleotide sequences which either enhance or repress transcription by RNA polymerases. Using band-shift assays, we found that the migration of a 3' fragment of GAP-43 mRNA on non-denaturing gels was retarded in the mRNA isolated from cells undergoing neurite outgrowth. Factors from undifferentiated cells caused the mRNA fragment to migrate to a position identical to these binding proteins, cellular proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with a radiolabeled fragment of the 3' end of GAP-43 mRNA. Northern blot analysis of differentiating cells with high GAP-43 mRNA binding activity; this protein did not bind control RNA and was barely detectable in unstimulated PC12 cells. Another GAP-43 mRNA binding protein was visualized by similar methods in some types of non-promoter regulatory sequences from other genes (e.g. Cell 32, 503). The 1 kb DNA fragment containing all of these functionally characterized elements also contains upstream of the promoter, a sequence motif common to some other non-promoter regulatory sequences from other genes (e.g. Cell 32, 503). The 1 kb DNA fragment containing all of these functionally characterized elements also contains upstream of the promoter, a sequence motif common to some other non-promoter regulatory sequences from other genes (e.g. Cell 32, 503). The 1 kb DNA fragment containing all of these functionally characterized elements also contains upstream of the promoter, a sequence motif common to some other non-promoter regulatory sequences from other genes (e.g. Cell 32, 503). Further, we have examined the effects of NGF and other agents that modulate neurite outgrowth on the steady-state levels and on the relative rates of decay of GAP-43 and other neuronal mRNAs. By Northern blot analysis, we have found that GAP-43 mRNA levels in PC12 cells were increased in response to the phorbol ester TPA, but not by either dibutyryl-cAMP or the Ca2+ ionophore A23187, suggesting that GAP-43 expression in PC12 cells depends on the activation of PKC. Furthermore, TPA had a much greater effect than NGF on both the onset and extent of process outgrowth and on the levels of the mRNAs for the neuronal proteins GAP-43, β-tubulin II (β-tub) and actin. In mRNA decay studies, TPA was found to increase 3-4 fold the half-lives of GAP-43, β-tub and actin mRNAs but had no effect on that of the house-keeping enzymes deoxyribonucleoprotein (C3PD). The effect of TPA on the stability of GAP-43 and β-tub mRNAs was blocked by dexamethasone but not by cycloheximide, suggesting that post-translational modification of a neuronal factor is required for these effects. In conclusion, our results indicate that in addition to transcriptional control, post-translational mechanisms may play an important role in the regulation of gene expression during neuronal outgrowth. Supported by NSF (BSF-R011199) and the American Paralysis Association (PBI-9006) to N.P.B.
525.13 POSSIBLE ROLE OF CALMODULIN BINDING TO B-50 (GAP-43) IN THE MECHANISM OF NORADRENALINE RELEASE.

B-50 (also known as GAP-43, F1 and neuroginin) is a neuron-specific protein kinase C (PKC) substrate and is thought to be involved in the mechanism of noradrenaline release (Dekker et al., 1989). In purified systems and in native synaptosomal plasma membranes it has been shown that B-50 binds calmodulin in the absence but not in the presence of calcium. In this study we investigated whether B-50/calmodulin interaction is involved in the mechanism of noradrenaline release. Calcium-induced NA release from Sympotinstio-O-pretreated synaptosomes was measured using [H-NA]. B-50/calmodulin interaction was measured using the homobifunctional crosslinker diiminthidilisulphate (De Graan et al., 1990). GAP-43, 52.139, 61,381, and 61,504 were the EC50 for complex dissociation and calcium-induced NA release at 10(-6)M in the presence or absence of 150 mM K+.

525.15 IDENTIFICATION OF THREE PHOSPHORYLATION SITES IN THE GROWTH-ASSOCIATED PROTEIN GAP-43.
S.A. Spencer and M. Willard.

The identification of specific phosphoamino acids in purified GAP-43, a protein that is concentrated in neuronal growth cones and certain perinatal brain regions, has been suggested to perform functions in axon outgrowth, transmitter release, and synaptic plasticity. It is a substrate for protein kinase C, which phosphorylates it at a single site, Ser 41, and thereby changes its affinity for calmodulin. Previous experiments indicated that in cultured neurons, GAP-43 is phosphorylated at additional sites by other protein kinases. To identify these additional sites, dispersed superior cervical ganglion cells (from rat were grown in culture for five days and then labeled with 32P-orthophosphate for four hours. GAP-43 was immunoprecipitated from these cultures and then proteolyzed with trypsin or endoproteinase Asp-N; the resulting radioalbeleed peptides were purified by reverse-phase HPLC. Sequential Edman degradation of these phosphorylated fragments produced three sequences corresponding to amino acids 38-43, 90-96, and 167-176 of GAP-43. The first peptide, IQASFR, was phosphorylated at site 41, the site identified by previous studies. The second and third peptides at the cycles of Edman degradation corresponding to Serine 94 and threonine 172 respectively, demonstrating that these two sites, in addition to site 41, are phosphorylated in cultured neurons. The similarity of the amino acid sequences surrounding these two sites, i.e. APAT S'**PKA, and AAP T**PAE, suggests that they may be phosphorylated by the same enzyme. These sequences resemble those recognized by a recently described enzyme designated proline-dependent proline kinase. (Supported by NIH grant EY02832).

525.16 GAP-43 mRNA EXPRESSION IN THE RAT HIPPOCAMPUS.
H.M. Chao and B.S. McEwen.

GAP-43 is a neuron-specific protein that is found in growth cones of axonal and dendritic processes. It is a substrate for protein kinase C, which phosphorylates it at a single site, Ser 41, and thereby changes its affinity for calmodulin. Previous experiments indicated that in cultured neurons, GAP-43 is phosphorylated at additional sites by other protein kinases. To identify these additional sites, dispersed superior cervical ganglion cells (from rat were grown in culture for five days and then labeled with 32P-orthophosphate for four hours. GAP-43 was immunoprecipitated from these cultures and then proteolyzed with trypsin or endoproteinase Asp-N; the resulting radioalbeleed peptides were purified by reverse-phase HPLC. Sequential Edman degradation of these phosphorylated fragments produced three sequences corresponding to amino acids 38-43, 90-96, and 167-176 of GAP-43. The first peptide, IQASFR, was phosphorylated at site 41, the site identified by previous studies. The second and third peptides at the cycles of Edman degradation corresponding to Serine 94 and threonine 172 respectively, demonstrating that these two sites, in addition to site 41, are phosphorylated in cultured neurons. The similarity of the amino acid sequences surrounding these two sites, i.e. APAT S'**PKA, and AAP T**PAE, suggests that they may be phosphorylated by the same enzyme. These sequences resemble those recognized by a recently described enzyme designated proline-dependent proline kinase. (Supported by NIH grant EY02832).
AUTORADIOPHIC PATTERNS OF GAP-43 mRNA EXPRESSION IN ADULT RAT BRAIN. L. Kruger, Dept. of Anatomy and Cell Biology, UCLA Medical Center, Los Angeles, CA; C. Bendotti, R. Rivolta, and J. Samadilic, Lab. Neuropharmacology, Mario Negri Institute, Milan, and M. Bontempoglio, University of Verona, Italy.

The neuron-specific protein associated with axonal outgrowth during development and nerve regeneration, GAP-43, is expressed in highly selective neuronal populations in the adult rat brain. We previously reported GAP-43 mRNA expression in mature brainstem monoaminergic neurons (Bendotti et al., '91). A survey of the distribution of GAP-43mRNA throughout the adult CNS revealed higher expression to localizations that differ from the distribution of immunoreactivity reported by others.

High mRNA levels are evident in the primary olfactory and in the secondary gustatory pathways, although "secondary," midline and intralaminar thalamic n. exhibit high levels. In cerebellar cortex, insular and parietal fields, high expression is evident. Other regions of high density include inferior olive and cerebellar granular layer, hippocampus CA3, entorhinal cortex, amygdala, hypothalamic n., bed n. of stria terminalis, n. basalis, lateral reticular, peripedalucal gray, and substantia gelatinosa. The variety of anatomical substrates provides a new basis for examining the propensity for synaptic "plasticity" in mature neurons.

SPECIFICITY OF NGF-INDUCED CEREBROVASCULAR NEURONS OF THE BASAL FOREBRAIN. We have now tested the specificity of NGF-induced cerebrovascular axons in the adult rat brain. We have previously shown that intraventricular infusion of nerve growth factor (NGF) into the adult rat brain elicits sprouting from mature cerebrovascular axons associated with the intracranial internal carotid artery (ICA). A 3-fold increase in the total number of perivascular axons was found at the ultrastuctural level (Isaacson et al., 1990). Since the ICA receives innervation from sympathetic, sensory, and parasympathetic sources, we undertook the following experiments to determine: 1) whether the sprouted axons are sympathetic in origin and 2) whether non-sympathetic fibers respond to NGF in the absence of sympathetic fibers. One group of rats received bilateral superior cervical gangliectomy (sxG). Two weeks later, the group received an infusion of NGF (NGFsx) or cytochrome C (VEHsx) and was sacrificed 3 days later. The other group received gangliectomy 3 days prior to the onset of the infusion and was sacrificed at the end of the 14-day infusion period (sxNGF or sxVEH). The total number of perivascular axons associated with the ICA was determined by immunocytochemistry. Bilateral gangliectomy resulted in a significant (73%) reduction in the number of perivascular axons in intact sympathetic nerves. This decrease was reversed by NGF although a decrease in perivascular axons in sxNGF rats (47%) was noted, and the decrease was greater in sxVEH rats (67%) than in sxNGF rats (47%). The results indicate that the mRNAs for NGF-R and GAP 43 are induced in the damaged fibres shows no such requirement (Diamond et al., 1987, PNAS 84:6596). In our approach to explain this phenomenon, we have begun to look at the expression of NGF-R and GAP 43 mRNA.

Changes in NGF Receptor and GAP 43 mRNA Associated with Collateral Sprouting and Regeneration of Dorsal Cutaneous Nerves in the Rat. K. M. Moseley, B. D. Ross*, A. Glotzer, M. Holmes* and J. Diamond, Dept. Biomedical Sciences, McMaster University Medical Centre, Hamilton, Ontario, Canada.

Previous studies have demonstrated that collateral sprouting of intact nociceptive nerves is NGF-dependent, while regeneration of the damaged fibres shows no such requirement (Diamond et al., 1987, PNAS 84:6596). In our approach to explain this phenomenon, we have begun to look at the expression of NGF-R and GAP 43 mRNA. DRGs of regenerating, collateral sprouting and control dorsal cutaneous nerves were isolated and used for Northern blot hybridization (ISH) to examine changes in mRNA distribution at the cellular level. These changes were quantitated by silver staining following total RNA and PCR amplification of the specific messages. The results indicate that the mRNAs for NGF-R and GAP 43 are elevated in the DRGs of collateral sprouting nerves at 1 day post-op, decreasing to control levels by 1 week post-op. The ISH results show that these changes are specific to the small DRG neurons. We are presently using the same approach to study "precocious sprouting" (e.g. Doucette & Diamond, 1987, J. Comp. Neurol. 261:592), a dramatic shortening in the latency of nociceptive collateral sprouting induced by increased activity. (This work is supported by the Centres of Excellence for Neural Regeneration and Functional Recovery.)

ANTII-NGF TREATMENT BLOCKS THE COLLATERAL SPROUTING OF CHOLINERGIC FIBERS IN THE HIPPOCAMPUS. L.G. Isaacson. 1 Dept of Zoology, Miami University, Oxford, OH 45065, 2 Dept of Neuromuscular, University of Cincinnati College of Medicine, Cincinnati OH 45267.

We have previously shown that intraventricular infusion of nerve growth factor (NGF) into the adult rat brain elicits sprouting from mature cerebrovascular axons associated with the intracranial internal carotid artery (ICA). A 3-fold increase in the total number of perivascular axons was found at the ultrastuctural level (Isaacson et al., 1990). Since the ICA receives innervation from sympathetic, sensory, and parasympathetic sources, we undertook the following experiments to determine: 1) whether the sprouted axons are sympathetic in origin and 2) whether non-sympathetic fibers respond to NGF in the absence of sympathetic fibers. One group of rats received bilateral superior cervical gangliectomy (sxG). Two weeks later, the group received an infusion of NGF (NGFsx) or cytochrome C (VEHsx) and was sacrificed 3 days later. The other group received gangliectomy 3 days prior to the onset of the infusion and was sacrificed at the end of the 14-day infusion period (sxNGF or sxVEH). The total number of perivascular axons associated with the ICA was determined by immunocytochemistry. Bilateral gangliectomy resulted in a significant (73%) reduction in the number of perivascular axons in intact sympathetic nerves. This decrease was reversed by NGF although a decrease in perivascular axons in sxNGF rats (47%) was noted, and the decrease was greater in sxVEH rats (67%) than in sxNGF rats (47%). The results indicate that the mRNAs for NGF-R and GAP 43 are induced in the damaged fibres shows no such requirement (Diamond et al., 1987, PNAS 84:6596). In our approach to explain this phenomenon, we have begun to look at the expression of NGF-R and GAP 43 mRNA. DRGs of regenerating, collateral sprouting and control dorsal cutaneous nerves were isolated and used for Northern blot hybridization (ISH) to examine changes in mRNA distribution at the cellular level. These changes were quantitated by silver staining following total RNA and PCR amplification of the specific messages. The results indicate that the mRNAs for NGF-R and GAP 43 are elevated in the DRGs of collateral sprouting nerves at 1 day post-op, decreasing to control levels by 1 week post-op. The ISH results show that these changes are specific to the small DRG neurons. We are presently using the same approach to study "precocious sprouting" (e.g. Doucette & Diamond, 1987, J. Comp. Neurol. 261:592), a dramatic shortening in the latency of nociceptive collateral sprouting induced by increased activity. (This work is supported by the Centres of Excellence for Neural Regeneration and Functional Recovery.)


In this report we describe the development and characterization of a polyclonal chicken antibody against the recombinant extracellular domain of the human NGF. This paradigm offers several distinct advantages. First, the evolutionary differences of antigen source and antibody producer enhance antigenic potential. Second, egg-yolk immunoglobulins are readily accessible, produced in large quantities, and conveniently packaged for facile storage. The purified antibody is novel in its ability to recognize receptor from a variety of species (including human, rat, mouse, and guine pig), as determined by immunocytochemistry, immunoprecipitation and/or in vivo retrograde transport assays. Furthermore, this antibody has the capacity to block ligand binding to these receptors. To date this has only been demonstrated for the anti-human NGF monoclonal antibodies, e.g. ME20.4-1Gg. As a result of the broad range of species reactivity we propose that this antibody will be useful in determining the role of the low-affinity NGF in signal transduction, as well as contributing to improved receptor immunosays (e.g., electron microscopy and immunoblotting).
NEWBORN RATS INDUCES AN INCREASE OF ACETYLCHOLINESTERASE (AChE) ACTIVITY IN FOREBRAIN REGIONS. G. Vantini, INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF NGF TO ADULT RATS RESULTS IN A PROLIferATION OF NEURONS IN THE HIPPOCAMPUS. A. Monti, J. H. Kordower, and J. R. Williams, Dept. of Physiology, Univ. of Michigan, Ann Arbor 48109 and The Upjohn Co., Kalamazoo, MI 49001.


INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF NGF TO ADULT RATS RESULTS IN AN INCREASE OF ACETYLCHOLINESTERASE (AChE) ACTIVITY IN FOREBRAIN REGIONS. A. M. O. T. Monti, J. H. Kordower, and J. R. Williams, Dept. of Physiology, Univ. of Michigan, Ann Arbor 48109 and The Upjohn Co., Kalamazoo, MI 49001.


NGF ENHANCES CHOLINERGIC TRANSMISSION BETWEEN NUCLEUS BASALIS AND BASOLATERAL AMYGDALA IN RAT. H.C. Moore, P.R. Binnie, M.S. Washburn and L.R. Williams, Dept. of Anatomy, Univ. of Illinois Sch. Med., Chicago, Ill.

Published work on the effects of antibodies to Nerve Growth Factor (ANTI-NGF) treatment on rats has shown an increase in the number of unmyelinated central processes of dorsal root ganglia (DRG) neurons. This increase is thought to be due to the sprouting of small peripheral DRG processes. We have quantified the number of peripheral DRG processes in the peripheral nerves of ANTI-NGF treated rats compared to untreated rats following a selective nerve injury to eliminate the peripheral and sympathetic fibers. We report the numbers of peripheral processes in an NGF deprived environment decrease by 48% compared to untreated controls and the decrease is selective for the unmyelinated fiber population. Since the majority of the unmyelinated population is nociceptive, two nociceptive behavioral measures, one reflexive (tail flick) and one nonreflexive (paw or skin pinch), were performed and demonstrated decreased response times in the ANTI-NGF treated compared to untreated and proinflammatory treated rats. These data suggest a direct effect primarily on the unmyelinated sensory population which results in altered nociceptive behavior, induced by the suppression of one endogenous factor, NGF. Supported by NS11351, NS01127, HL07615, Bristol Myers, Hall Endowment, Sigma Xi.


The influence of NGF on the development of cholinergic striatal interneurons in terms of cell number, cell size and complexity of dendritic tree was studied in vitro. The striatum was taken from newborn Wistar rats and chopped in 250 μm thick slices. From these slices identical circular pieces of 1.1 mm in diameter were punched out and 4 series of 20 cultures each were grown in vitro using the roller tube method. The mean of the 4 culture series contained 5 ng/ml NGF, 10 ng/ml NGF plus 50 ng/ml anti-NGF antibody (AB) or 50 ng/ml NGF plus 50 ng/ml anti-NGF-AB respectively. After 13 days in vitro all cultures were fixed and stained for AChE. The number of AChE positive interneurons in cultures grown with NGF, without NGF, with anti-NGF-AB or with NGF plus anti-NGF-AB was 12.7 ± 10.2, 6.5 ± 14.5, 4.4 ± 14.5, respectively. The mean soma profile area of the cholinergic neurons in cultures grown with NGF, without NGF and with NGF plus anti-NGF-AB were 768 ± 102, 480 ± 151 and 441 ± 158 μm2, respectively. 5 cells each of cultures grown with and without NGF were reconstructed and the dendritic trees analyzed. The mean combined dendritic length of the cells grown with and without NGF was 5751 ± 228, 480 ± 151 and 441,8 ± 158, respectively. The mean number of stem dendrites was 5.6 ± 1.9 and 4.2 ± 0.4, respectively and the mean total number of branch points per cell was 47 ± 2.9 and 28 ± 5, respectively. The areas covered by the dendritic tree of the neurons grown with and without NGF were not different. These data demonstrate first, that NGF promotes survival and growth of the cholinergic striatal interneurons in culture and second, that cells of the same age in vitro grown with NGF possess a complex dendritic tree than those grown without NGF.


Nerve growth factor (NGF) is a potent neurotrophic agent required for the differentiation and maintenance of neural crest-derived neurons. The PC12 rat pheochromocytoma cell line provides a useful model for examining mechanisms by which NGF induces cellular differentiation. NGF exposure causes these cells to adopt a neuron-like phenotype. This trans-differentiation is associated with stimulated expression of several neuroepithelial genes including galanin. The induction of galanin expression in these cells is rapid, peaking 4 hours after NGF addition, with mRNA increased more than 100-fold. To study the transcriptional regulation of galanin by NGF, galanin reporter constructs were transiently transfected into PC12 cells by transient expression in PC12 cells. Detection analysis revealed 2 regions in the galanin 5'-flanking sequence that conferred NGF responsiveness to the transfection system. DNA-binding activity of gel shift analysis using nuclear protein extracts from normal and NGF-treated cells was found to be specific for the galanin proximal promoter region by using radiolabeled double-stranded galanin DNA-protein complexes as probes. EMSA analysis revealed consensus binding sites for AP1, AP2, AP3, NF1, SP1 and zif-268. Efforts are underway to isolate and further characterize the protein that binds to this NGF-responsive element.


Nerve growth factor (NGF) and nerve growth factor receptor (NGF-R) were immunolocalized in the peripheral auditory system of 5 day postnatal rats using gold particle labeled antibodies detected in both the inner (IC) and outer (OHC) hair cells of Corti's organ, the soma as well as peripheral and central axons of spiral ganglion neurons and in the Schwann cells of the VIIIth cranial nerve. NGF-R antibody was not immunolocalized over the somas of either the IHC or OHC, however intense immunolabel was present over the neurons that compose the spiral ganglion and nerve endings at the bases of both the IHC and OHC. Explants of 3PF rat organ of Corti with attached spiral ganglia and antibody were processed for in situ localization. 2.5 ng NGF can disrupt the normal pattern of innervation (detected by anti-neurofilament antibodies) when compared to normal serum controls. These findings strongly support a role for NGF or a member of the NGF family (i.e. BDNF or NT3) in development of the peripheral auditory system.

Supported by grants from NIMH DCO0088 to TRL and the National Fund for Scientific Research of Belgium and the Foundation Medecale Reine Elisabeth to OM and PFL.

NGF ATTENUATES NBMB LESION-INDUCED CHOLINERGIC NEOUROCHEMICAL MARKER DEFICITS IN YOUNG, BUT NOT IN AGED, F344 RATS. A.C. Sarnucci, P.O. Kalafat, and V. Zarate. Psychiatry Service, Bronx VAMC & Dept. of Psychiatry, M. Sinal School of Medicine, New York, NY 10468.

This study's aim was to determine whether nerve growth factor (NGF) administration would enhance cholinergic activity in central nervous system areas that have sustained experimentally-induced cholinergic lesions. Accordingly, 14 young (3-4 months) and 13 aged (22-23 months) F344 rats were prepared with NMDA-induced unilateral lesions of the NBM. One week after the surgical session, subjects were implanted with a subcutaneously placed osmic pump filled either with 6 μg of 8 NGF (young, n=7; aged, n=6) or artificial cerebrospinal fluid (young, n=7; aged, n=6) and continued on an inducing cannula directed at the lateral ventricle. Animals were sacrificed and their brains dissected two weeks after surgery. Concentrations of CAT and AChE within the caudate, cortex and hippocampus were measured. Results indicated a significant lesion-induced depletion of CAT and AChE within the frontal cortex of both young and aged subjects (p<.001). Nerve growth factor attenuated these depletions by approximately 35%. However, this effect of NGF was observed only in young animals (p<.05). This finding contrasts a previous report from our laboratory indicating NGF's ability to increase cholinergic neurochemical markers in aged non-lesioned animals. Taken together, these data suggest that NGF's effect on cholinergic activity in the aged brain may depend upon the neurochemical integrity of the tissue.

Supported by a grant from the American Federation for Aging Research.

NERVE GROWTH FACTOR REGULATES THE EXPRESSION OF THE CALCIUM-BINDING PROTEIN S100A. D.B. Zimmer, B.H. Cochran and S.J. Strada. Dept. Pharmacol., Univ. S. Alabama, Mobile, AL 36688. One mechanism by which nerve growth factor (NGF) induces differentiation in neuronal cells is to increase the expression of specific proteins such as 42C, a member of the S100 family of calcium-binding proteins. This study utilized PC12 cells to examine the effect of NGF on the expression of other members of this protein family, S100A and S100B. Northern blot analysis revealed that NGF increased the expression of S100A mRNA in a dose-dependent manner. This increase was obtained by a 2-fold increase in S100A protein in NGF-treated cells. While, NGF treatment resulted in increased S100B protein levels, it did not alter the S100A protein binding profile or subcellular distribution of S100A. In both treated and untreated cells, a diffuse punctate staining pattern in the cell body with no detectable staining in the cell processes. Altogether, these results demonstrate that NGF increases S100A expression and suggest that NGF may mediate cellular responses to NGF in neuronal cells.
Nerve Growth Factor Signal Transduction in Rat Septal Neurons

G.W. Varillic*, G.A. Nett, B. Antisdel* and N.J. Fantazir. Deps. of Internal Medicine and Anatomy, Univ. of Iowa College of Medicine, Iowa City, IA 52202.

Although nerve growth factor (NGF) has been detected in the gastrointestinal tract of adult rats (Wescamp and Otten, J. Neurochem., 48:1779, 1987), the cells(s) responsible for NGF synthesis in the tract have not been identified. Since intestinal epithelial cells are a major cellular component of the gastrointestinal tract, the aim of this study was to determine whether intestinal epithelial cells are a potential source of NGF. Two cell lines, CaCO-2 and IEC-6, were selected for study since they are derived from intestinal specimens and display many characteristics of epithelial stem cells. Conditioned medium (CM) was obtained from confluent cultures of CaCO-2 and IEC-6 and examined in two NGF bioassays. CM from both cell lines induced neurite outgrowth in pheochromocytoma (PC12) cells and sensory neurons (dorsal root ganglia). This outgrowth was similar to that induced by purified NGF and was inhibited by polyclonal anti-NGF antibody. In addition, an NGF-ELISA (Mobley et al., Neuros. 3.655, 1989) detected NGF in CM from CaCO-2 and IEC-6 cells. These data demonstrate that CaCO-2 and IEC-6 cells synthesize and release NGF into the culture medium. This suggests that intestinal epithelial cells are a potential source of NGF in the gastrointestinal tract. Supported by the CFCA, Univ. of Iowa College of Medicine Grant, and Univ. of Iowa Aging Research Seed Grant.

NGF Receptors in the Olfactory System

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To address the possible role(s) of NGF in development and regeneration in olfactory system, we have assessed the cellular localization of NGFRs in adult and developing rats using two monoclonal antibodies directed against NGFRs (217C and 192). NGF-immunoreactivity (NGFR-IR) was observed on fibers in deep layers of the olfactory bulb late in development but was not apparent before E18. NGFR-IR was also observed in neurons of the basal forebrain and their axons coursing into the deep layers of the olfactory bulb. In the adult, NGFR-IR remains abundant in olfactory bulb glomeruli and in fibers in deeper layers of the bulb. These results suggest that NGFRs are expressed early in the development of the olfactory system. NGFRs in the adult forebrain cholinergic neurons with ibotenic acid; we predict that this manipulation will eliminate NGFR from the adult olfactory bulb. In developmental tissue there was high NGFR-IR in the epithelium and olfactory nerve which may be localized to Schwann cells. However, in contrast to peripheral animals, only a few streaks of NGFR-IR were present in the adult olfactory nerve. These "streaks" of NGFRs seen in the adult nerve may represent fascicles transiently "dexterated" by normal olfactory neuron turnover and replacement. Consistent with this hypothesis, NGFRs were robustly re-expressed in the olfactory nerve following lesion of the olfactory epithelium [Supported by NIDCD DC00347 and NINDS NS29218].

Hormones and Development: Steroid Receptors

527.1 Ontogeny of the Hippocampal Binding Capacity of the Glucocorticoid and Mineralocorticoid Receptor.

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The developing HPA axis is hyporesponsive to stressful stimuli during early postnatal development. One mechanism by which this occurs is the failure to terminate the corticosterone rise is evident following certain stressors. The hippocampal (HC) glucocorticoid (GR) and mineralocorticoid (MR) receptors (R) are involved in the modulation of the HPA axis through negative feedback. In order to investigate the emergence of this mechanism, we have studied the ontogeny of GR and MR binding capacity (Bmax) in the HC. Adrenalectomy (ADX) was performed on day: 6, 10, 14, 18, 22, 28, 35, 45 and adult animals. GR and MR Bmax HC analysis was done 12 hours after ADX using saturating amounts of [3H]corticosterone. R12608, a GR agonist and corticosterone that also binds to GR, was used to calculate specific GR or MR Bmax. GR Bmax reached adult levels on day 10 and remained steady. In contrast, MR Bmax was greater than adult from day 6 until day 45; when it decreased and approached adult concentrations. Thus, GR/MR Bmax ratios increased with age (days 1-1 to adult-61). Looking at the total HC, it was evident that the greatest absolute increase for both receptors occurred between days 22 and 45, at a time when failure to terminate the adrenocortical response is described. This suggests that an impaired glucocorticoid modulation during development is not the result of lower GR and MR adrenocortical responses. The idea that GR can be cROSes to the receptor is consistent with the observed effects described above. The relative increase in GR/MR Bmax ratio during development is likely to be due to a variety of factors, including differences in receptor turnover rates and/or a change in the sensitivity of the GR/MR system to corticosterone. These results suggest that the GR/MR Bmax ratio changes may be indicative of the developing HPA axis and that the development of the HPA axis is a critical factor in the regulation of the HPA axis during early life. Supported by NIMH 4362923.

527.2 Developmental vs. Activational Effects of Gonadal Steroids on the Binding Parameters of Corticosteroid Receptors in Hippocampus of Male vs. Female Rats.

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We have previously reported that female rats have fewer hippocampal Type I and Type II corticosteroid receptors than do males, and that the in vitro affinity of the Type I receptor from females is several fold less than that from males [Brain Res.: 748:379, 1996]. In this study, we asked whether the presence of the gonads during development contributed to these differences or whether the short term presence of gonadal steroids is solely responsible. Male and female adult rats were adrenalectomized either on day 1 or 60 days of age. Male rats had been injected with either vehicle alone or to increase testosterone propionate for 10 days. Likewise, females were injected with vehicle or 2.5 mg estradiol benzoate. Animals were decapitated at 12 hr before sacrifice. For each hippocampus, Type I binding was measured using a saturation plot (1H-deamethasone + RU 38622) and total binding was measured with a single, saturating concentration of [3H]dehydrocorticosterone. Scatchard analysis of variance showed a main effect of sex steroids on the Brain of Type I receptors (p < 0.01): estrogen treated females had fewer Type I receptors than did the other groups. No significant effects were found for any sex steroids with regard to the Kd of the Type I receptor; values were similar to those previously reported for males. Type II receptor number showed a main effect of sex (p < 0.02); female groups had fewer Type II receptors than the males. Consistent with the presence of estrogen down-regulates the Type I receptor but does not influence its affinity. In contrast, expression of the Type II receptor may be developmentally influenced by gonadal steroids. Supported by NS 22130.
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cannabinoids are required for normal CNS development. High levels are neurotoxic and reduce myelination. The actions of cannabinoid receptor agonists and antagonists are mediated via different affinity Type I (cannabinoid) receptor or a low affinity Type II (glutamate) receptor. MRNA and immunoreactivity (ir) of both receptors have been localized in the adult rat cerebellar cortex, a region which undergoes most of its development during postnatal life. We have studied the development of Type I-ir and Type II-ir in the postnatal rat cerebellar cortex using MINREC4 antiserum and BUGR2 monoclonal antibody, respectively. Type I-ir Purkinje cells were first observed at postnatal day 3 (P3) in contrast to Type II-ir cells which were first observed at P15. In the Purkinje cell layer, both receptor systems developed rapidly to adult levels by P20. Type I-ir and Type II-ir in the granular and molecular layers were first observed at P5 and P15 respectively; however, the rate of development was slower. By P30 the density of immunoreactive cells in these layers was still less than in the adult. The earlier development of the Type I receptor system suggests it may mediate most of the actions of cannabinoids in the cerebellum during early postnatal life. Supported by NS24148.

S27.5

COCAINE DECREASES HYPOTHALAMIC ESTROGEN RECEPTOR MRNA LEVELS IN NEONATAL FEMALE RATS. C. Benton*, R. Hahnabadi, N.J. Haugen, E. Bhasin* and D.H. Glazer. *Center for Endocrinology, Harbor/UCLA Medical Center, Torrance, CA 90509.

Neonatal exposure to cocaine or estradiol (E) androgenizes the rat brain. We have previously shown that cocaine interferes with this process by reducing hypothalamic nuclear estrogen receptor (ER) binding. To examine if these effects of cocaine are mediated via changes in hypothalamic ER mRNA, we examined day old female rats. Rats were reared and tested as described previously. 

ER mRNA levels were quantified by hybridization of Northern blots with a human ER cDNA probe. E treatment increased levels of hypothalamic ER mRNA; this effect was blocked by cocaine. Thus, cocaine antagonizes E-stimulation of ER mRNA in the hypothalamus, perhaps by influencing ER mRNA transcription or stability. This may result in fewer ERs available for interaction with DNA-responsive elements, and in this manner interfere with activation of E-responsive genes involved in androgenization. (Supported by NIH DK 05751, DA 04490 and HD 23483.)

S27.7


Glucocorticoids are required for normal CNS development. High levels are neurotoxic and reduce myelination. The actions of glucocorticoid receptor agonists and antagonists are mediated via different affinity Type I (mineralcorticoid) receptor or a low affinity Type II (glucocorticoid) receptor. MRNA and immunoreactivity (ir) of both receptors have been localized in the adult rat cerebellar cortex, a region which undergoes most of its development during postnatal life. We have studied the development of Type I-ir and Type II-ir in the postnatal rat cerebellar cortex using MINREC4 antiserum and BUGR2 monoclonal antibody, respectively. Type I-ir Purkinje cells were first observed at postnatal day 3 (P3) in contrast to Type II-ir cells which were first observed at P15. In the Purkinje cell layer, both receptor systems developed rapidly to adult levels by P20. Type I-ir and Type II-ir in the granular and molecular layers were first observed at P5 and P15 respectively; however, the rate of development was slower. By P30 the density of immunoreactive cells in these layers was still less than in the adult. The earlier development of the Type I receptor system suggests it may mediate most of the actions of corticosteroids in the cerebellum during early postnatal life. Supported by NS24148.

S27.6


In this study, we have used the Brazilian short-tailed opossum (Monodelphis domestica) as a model to study the development of estrogen receptors in the mammalian brain. Monodelphis is a small, pouchless marsupial which breeds well under laboratory conditions and whose young are born in an immature sexually undifferentiated state. The Abbott H222 monoclonal estrogen receptor antibody (gift of Abbott Laboratories) was utilized in an indirect immunohistochemical procedure to detect estrogen receptors in the developing opossum brains. Estrogen receptors were first expressed in the dorsomedial and ventromedial hypothalami of the opossum ten days after birth (10PN). Most regions that contained estrogen receptor immunoreactivity (ER LI) in the adult opossum contained ER LI at 15PN. These areas include the lateral septal, medial preoptic area, bed nucleus of the stria terminalis, periventricular preoptic area and hypothalamus, amygdala, dorsomedial and ventromedial hypothalamic nuclei, arcuate nucleus, ventral premammillary nucleus, and the midbrain central grey. The number of ER LI cells increased from 15PN-60PN in all regions of the brain that contain ER LI cells in the adult opossum. The time course of expression indicates that estrogen receptors are present in early development of the Monodelphis brain and may mark the beginning of a critical period for sexual differentiation of the opossum brain.

S27.8

THE OCCUPATION OF NUCLEAR ESTROGEN BINDING SITES IN THE FETAL MACAQUE HYPOTHALAMUS BY STEROIDS OF TESTICULAR ORIGIN. F.W. Bonsal, D. Zuppan* and R.P. Michael. Department of Psychiatry, Emory University School of Medicine and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

Previous results with macaque fetuses given [3H]-testosterone (3H-T) at about 120 days of gestation demonstrated a sex difference in nuclear levels of the aromatized metabolites 3H-E2 and 3H-E1. To determine if this might be due to prior occupation of receptors by endogenous steroids in the male, we have now compared the uptake of 3H-T and its metabolite in male and female macaques (T 571 ± 215.5 ng/100 ml, N = 5). The demonstration that nuclear binding of these metabolites to hypothalamus and amygdala was significant in the male, but not in the female, supports the hypothesis that aromatized metabolites of 3H-T do not differ in the 3 groups. Furthermore, the hypothesis of cell-specific occupation of binding sites in the hypothalamus and amygdala was supported by the finding that there was no sex difference in the uptake of 3H-E1 by hypothalamus and amygdala of macaques at any time of gestation. The results suggest that aromatized metabolites of 3H-T acting via estrogen receptors during fetal life could, therefore, account for some of T's organizational effects on primate physiology and behavior. Supported by USPHS grant MH 40620 and by the Georgia Department of Human Resources.

HORMONES AND DEVELOPMENT: STEROID RECEPTORS THURSDAY PM

THURSDAY PM
527.9 ONSET OF ANDROGEN RECEPTOR mRNA EXPRESSION IN THE CNS OF XENOPUS LAEVIS; LOCALIZATION USING IN SITU HYBRIDIZATION. M.A. Cooper, L.J. Dicker, B.D. Kelley, Dept. Biol. Sci., Columbus Univ., NY 10027

In the clawed frog, Xenopus laevis, male mate calling is controlled by testicular androgen secretion. Nuclei in the CNS calling circuit have been mapped; all have been shown to concentrate radiolabeled androgen, as does the effector organ, the larynx. Previous studies using intrathecally dihydrotestosterone detected CNS androgen-containing cells from tadpole stage 64 on; no labeled cells were detected at stage 60. The absence of labeled cells at stage 60 could be due to lack of androgen receptor (AR) expression or to competition for labeling by endogenous hormones. To determine the onset of AR expression in the calling nuclei, we localized AR transcripts in developing tadpole brains using in situ hybridization. A cloned AR cDNA probe, isolated from X. laevis laryngeal CDNA, was used. AR mRNA expression in neurons was detected as early as stage 56, when the gonads differentiate. At this and subsequent stages, transcripts were localized to motor nuclei of cranial nerves IX-X, medullary reticular formation, a presumed sensory nucleus of cranial nerve V, preglomerular nucleus of the dorsal segmental area of the medulla, and the nucleus of the fourth ventricle. Arborization of the anterior pituitary, ventral thalamus, and anterior spinal cord. The earliest stages of receptor expression and the hormonal control of this developmental program are under investigation.

Supported by NS 23684

527.10 DEVELOPMENTALLY REGULATED ECDYSTROID BINDING AND IMMUNOCYTOLOGICALLY IDENTIFIED INSECT NEURONS. E.D. Kropp, P.A. Stumpf, and B. Kemps, Dept. of Cell Biol. and Amat., UNC, Chapel Hill, N.C. 27599

To study the action of 20-hydroxyecdysone (20-HE) on the CNS of larval Manduca sexta, high affinity ecdysteroid binding sites (Kd ≤ 4nM) in cerebral ganglia were observed in vivo with 3H-p8-histerone A (PRA, a 20-HE agonist) or oxidized HPLC-purified PRA. Certain autoradiograms were immunostained for tyrosine hydroxylase or the pro-ecdysteroid receptor (PETH). Analysis of autoradiograms revealed stage-specific expression of ecdysteroid receptors (ER) in the CNS during the 4th and 5th larval stadia. During the 4th stadia, the number of cerebral neurons with ER peaked on day 2 and decreased steadily until day 0 of the 5th stadium. In the 5th stadium, the number of target neurons peaked at the times of the two ecdysteroid peaks. ER during both stadia were present in neurosecretory centers of the protocerebrum, including the proecdysterocortices, and in certain neurons of the subesophageal ganglion (SEG). The proecdysterocortices exhibited nuclear ER only during periods of active ecdysteroid synthesis by the proecdysteroid glands. Similar developmental fluctuations in numbers of neurons with ER occurred in the SEG, where several target neurons stained for tyrosine hydroxylase. The results demonstrate stage-specific ecdysteroid binding in neurons with identified peptide and neurotransmitter functions.

HORMONES AND DEVELOPMENT: CNS

528.1 ESTROGEN INCREASES PROENCEPHALIN mRNA EXPRESSION IN THE VMN OF JUVENILE MALE AND FEMALE RATS. Annette C. Segarra, Jesus A. Angulo and Bruce S. McEwen. The Rockefeller University, Laboratory of Neuroendocrinology, 1320 York Ave., New York, N.Y. 10021

Estrogens have been implicated in sexual differentiation of the brain and in the regulation of reproductive behavior in adult rats. Previous studies indicate that estrogen administration in adults increases proenkephalin mRNA expression in the VMN of female, but not male, rats (Roman et al., Brain Res. 1990; 536:63). We have determined pro-enkephalin mRNA expression in estrogen-treated juvenile male and female rats to investigate the developmental pattern of estrogenic regulation of enkephalergic neurons in the VMN. Rats were gadedestomatized at 16 days of age and treated with estradiol benzoate (40 μg/kg) or oil from day 21-23. Sections of the ventromedial hypothalamic nucleus (VMN) and paraventricular (PVN) were hybridized with a labeled antisense probe for the enkephalin promoter (König et al., 1991; Endocrinol. 112:2735) in the VMN of juvenile and adult male rats. It is possible that the testosterone surge during puberty renders the male VMN unresponsive to estrogenic induction of enkephalinergic message (WS 07080 and 07081).

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528.2 LOCALIZATION AND HORMONAL REGULATION OF THE c-raf-1 PROTO-ONCOGENE IN DEVELOPING RAT HYPOTHALAMUS. R.C. Whorf and S.A. Tobet, Dept. of Biochemistry, E.K. Shriver Center, MD 20742 & Prog. in Neuroscience, Harvard Medical School, Boston, MA 02115.

In studying sexual differentiation of the brain, we examined the expression of the proto-oncogene c-raf-1 (Raf-1). We have immunocytochemically and biochemically determined the localization of Raf-1 in embryonic rat brain regions and its regulation by hormonal stimulation of the pituitary-adrenal system. In adult rats, we found the highest levels of Raf-1, 2-fold (1.5-2.3) higher than male hypophysectomized (HPX) rat hypothalamic nuclei were studied by in situ hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Estrogen caused an upward shift in the number of target neurons peaked at the times of the two ecdysteroid peaks. ER during both stadia were present in neurosecretory centers of the protocerebrum, including the proecdysterocortices, and in certain neurons of the subesophageal ganglion (SEG). The proecdysterocortices exhibited nuclear ER only during periods of active ecdysteroid synthesis by the proecdysteroid glands. Similar developmental fluctuations in numbers of neurons with ER occurred in the SEG, where several target neurons stained for tyrosine hydroxylase. The results demonstrate stage-specific ecdysteroid binding in neurons with identified peptide and neurotransmitter functions.

528.3 IMMUNOCYTOCHEMICAL AND HISTOCHEMICAL ANALYSIS OF GONADOTROPIN RELEASEING HORMONE (GnRH), TYROSINE HYDROXYLASE (TH), AND CYCLOPHOSPHAMIDE (CP) ACTIVITY WITHIN THE SEPTAL, HYPOTHALAMIC, AND THALAMIC AREAS OF CHICKS SHOWING EARLY SEXUAL MATURATION. G. S. Paller*, M. N. Sizemore, and W. J. Rueppel. University of Maryland at College Park, MD 20742.

A modified, retractable Halasz knife was used to produce parasagittal cuts isolating the hypothalamus from lateral brain structures in chicks, exposed to short days, during two weeks of age. By five weeks of age, operated chicks which showed advanced development of secondary sex characteristics were selected and placed in sham-operated controls prepared for immunocytochemical analysis of brain tissue. Three nuclei showed significant differences between treated and control birds as determined by at least two antisera from R. Benoit. The bed n. of the pallial commissure (nCPa), the paraventricular n. (PVN), and the anterior lateral thalamic n. (ALT). A greater number of GnRH perikarya were observed in the brains of treated birds as compared to sham-operated controls. A greater number of GnRH neurons were absent in both the TH and CP stained areas. The PVN of experimental birds had densely staining cells when compared to controls. The results demonstrate that the testis but not testosterone contribute to this photoperiod effect, males reared in short days (10L:14D) were castrated at 12.5 and 10 days) and implanted with a blank or TH-filled silastic capsule (5 mm i.d.) at the age of 5, as well as compared treated males reared in long days (16L:8D; n ≥ 15) served as control. Brain sections (60 μm) from the MPOA and DBB were processed for GnRH immunohistochemistry. A significant expression was detected in the MPOA and DBB of treated females, whereas none was detected in the MPOA and DBB of sham-operated females.


Pregnancy in the Djungarian hamster (age 25 d) is characterized by gonadotropin secretion and increased numbers of unipolar but not bipolar GnRH-immunoreactive (GnRH-i) cell bodies in the medial preoptic area (MPOA) and diagonal band of Broca (DBB), regions that contain the majority of GnRH perikarya. The suppression of GnRH-i cell number can be blocked by exposure to short days (Endo 1989:124 Suppl.257A, 1989). To determine whether the testes or testosterone (T) contribute to this photoperiod effect, males reared in long days (10L:14D) were castrated at 12.5 and 10 days and implanted with a blank or T-filled silastic capsule (5 mm i.d.) at the age of 5, as well as compared treated males reared in long days (16L:8D; n ≥ 15) served as control. Brain sections (60 μm) from the MPOA and DBB were processed for GnRH immunohistochemistry (R.L. and C.P. Hamer). A significant expression was detected in the MPOA and DBB of treated females, whereas none was detected in the MPOA and DBB of sham-operated females.

Differences in the number of GnRH cells in the MPOA were observed in either the brainstem or cortex. Administration of TP 3h after birth did not change Raf-1 levels examined 24h later. In summary, Raf-1 was localized within selective regions of the rat brain and its expression altered by exogenous prenatal hormonal stimulation. Raf-1 signal transduction may act to deform hypothalamic critical periods in sexual differentiation of the brain.

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528.5

SEX DIFFERENCES IN SYNAPTIC NUMBERS IN THE BINOCULAR AREA OF THE VISUAL CORTEX OF THE RAT. Silvia N. M. Reid and Janice M. Juraska. Neuroscience Program and Department of Psychology, University of Illinois, Champaign 61820.

We have previously found that male rats have more neurons in the binocular area of the primary visual cortex (Oc1B) and a larger Oc1B than female rats (Reid & Juraska, Neurosci. Abst., 1990). In the present study we investigated sex differences in synaptic connections. Synaptic density of layer II-III of the Oc1B was estimated from 7 littersmate pairs of 90 day old Long-Evans hooded rats. The number and the length of synapses were obtained from 42 electronmicrographs (40,000X) for each hemispheres of each animal. Synaptic length was used as a correction factor in estimation of synaptic densities.

Our preliminary data show that synaptic densities, the average synaptic length and the synapse-to-neuron ratio were not significantly different between sexes. Because layer II-III of Oc1B in the male rat was larger, it contained more asymmetrical synapses and total synapses (20%) than that of the female rat. More animals will be added. Supported by NSF BNS 89-09164.

528.7


The nucleus preopticus medianus (POMn) is a sexually dimorphic nucleus that is critically involved in the hormonal activation of male copulatory behavior. The larger volume observed in adult males appears dependent upon circulating testosterone (Panzica, Viglietti-Panzica, Calcagni, Anestetn, Schachet & Baltanaz, 1987). The present study is an attempt to determine exactly when during normal development this nucleus becomes dimorphic. POMn was drawn under a projecting microscope in Nissl stained coronal sections (40um) from animals sacrificed at two weeks of age (n=3 per sex), three weeks of age (n=3 per sex), and six weeks of age (n=3 per sex). Areas were measured by computerized planimetry from consecutive serial sections (n=10 per animal) immediately anterior and at all levels of the final section in which the anterior commissure (CA) was observed, so chosen because the nucleus gradually increases in size caudally and is absent in the telencephalon. Our preliminary data show that synaptic densities, the average synaptic length and the synapse-to-neuron ratio were not significantly different between sexes. Because layer II-III of Oc1B in the male rat was larger, it contained more asymmetrical synapses and total synapses (20%) than that of the female rat. More animals will be added. Supported by NSF BNS 89-09164.

528.8


The higher vocal center (HVC), a region necessary for song production in zebra finches, contains more neurons in adult males (who sing) than in females (who do not sing). This dimorphism is regulated by early estrogen exposure and arises because new HVC neurons are added in greater numbers in juvenile males than in females. It is not known when these late-generated HVC neurons first become sexually dimorphic, and thus, whether estrogen promotes their production or survival. We gave males and females 3-h-thymidine on days 15 and 16 after hatching to label HVC neurons born on these days. Labeled neurons within HVC were then counted at 12, 13, and 39 days after the 3H-Thy regime. Neuronal labeling was not seen in HVC 2 days after 3H-Thy, implying that longer is needed for migration and/or neuronal differentiation. But, by 13 days after birth, new HVC neurons were already sexually dimorphic; the number of labeled HVC neurons being 4-5X higher in males than in females. This sex difference enlarged only slightly between 13 and 39 days after birth because a larger proportion of labeled HVC neurons was lost in females than in males during this time. However, this neuronal loss was not significant in either sex. Currently, we are counting labeled HCV neurons at other timepoints to determine if sex differences in their number emerge before these cells incorporate into HVC and interact with sexually dimorphic afferent or efferent targets.

HORMONES AND DEVELOPMENT: MOTOR NEURONS

529.1

DEVELOPMENT OF NEURONAL NUMBER IN PRIMARY AFFERENTS OF A SEXUALLY DIMORPHIC NEUROMUSCULAR SYSTEM IS ANDROGEN-DEPENDENT. A. Mills and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, University of Idaho, Bloomington, IN 47405.

The spinal nucleus of the bulbocavernosus (SNB) in the rat contains many more motoneurons in males than females. Androgens produce this sex difference by influencing normally occurring motoneuron death, but the site of androgenic action is unclear. Primary afferents have been previously shown to be involved in the normal development of spinal motor nuclei (Davis et al., 1983; Okado and Oppenheim, 1984). To investigate the potential role of afferents in the regulation of SNB motoneuron number, we studied the development and androgen dependence of the dorsal root ganglia (DRGs) that innervate SNB target musculature.

Male rats injected with WGA-HRP or Fluorogold into an SNB target, the bulbocavernous muscle, showed extensive labeling of the L6 and S1 DRGs. Neuronal counts were therefore restricted to these DRGs, and made in normal males (M) and females (F) from embryonic day (E)18 through postnatal day (P)10, the period during which SNB motoneuron number is established. Counts were also made in females treated with testosterone propionate (TP) on E16-E22, P1, P3, and P5, a regimen which results in complete masculinization of SNB motoneuron number. Counts of DRG neurons declined from E18 through P10, by which time males had more DRG neurons than females [L6/S1 total: M (N=3), mean=8650 ± 220; F (N=3), mean=7030 ± 310]. Androgen treatment of females masculinized the DRG neuron number at P10 (M;N=2; mean=8000 ± 470). These results suggest that the DRG can be masculinized by TP, a finding consistent with the hypothesis that primary afferents may be important in establishing the androgen-dependent sex differences in SNB motoneuron number. (Supported by NIH NS08917 to AM)

529.2

HORMONAL CONTROL OF DENDRITIC DEVELOPMENT IN SEXUALLY DIMORPHIC RAT SPINAL NUCLEI. L.A. Guldstein and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Dendritic development of motoneurons in the spinal nucleus of the bulbocavernosus (SNB) is biphasic and androgen dependent. During normal postnatal development, SNB dendrites grow exuberantly and then retract to mature lengths. In adult males castrated at one week old, SNB dendrites fail to grow beyond pre-castration lengths, but grow exuberantly in castrates treated with testosterone (T). To determine whether the growth involves the conversion of D to dihydrotestosterone (DHT) or estrogen (E), male rats were castrated on postnatal day (P)7 and given daily injections of either DHT or E. Dendritic length and soma size of all SNB motoneurons were measured by computerized planimetry from consecutive serial sections (n=7 per sex). Treatment with DHT reproducibly labeled with HRP were measured at P28 or P49. Results show that DHT, but not E, is as effective as T in supporting dendritic growth of SNB motoneurons, landing further support for the role of estrogen in the masculinization of sexually dimorphic spinal motor nuclei.

The dendritic development and androgen regulation of motoneurons in the dorsolateral nucleus (DLN) was then examined. Treatment with DHT and a smaller size of DLN motoneurons in normal males and in castrates treated with either T or oil vehicle were measured at P7, P28, P49 and P70. Like the SNB, dendritic growth in the DLN is androgen dependent. Unlike the SNB, dendritic growth in the DLN is monoestrogenic; the dendritic length of motoneurons increases more than 500% between P7 and P70. These results suggest that in addition to androgens, other factors may play a part in regulating dendritic development. Given that SNB and DLN motoneurons occupy different spinal locations and have different dendritic geometries, one factor may be the differential distribution of affrents to the two nuclei. (Supported by NIH NS24877)

The bulbocavernous (BC), levator ani, and sphincter muscles of gerbils are innervated by a sexually dimorphic, androgen-sensitive motoneuron pool located above the central canal in lamina X of lumbar spinal cord. This research investigated the role of postnatal gonadal steroids in the development of this unique motor system.

On the day of birth (P1) male gerbils were castrated or given sham surgeries. Female gerbils were injected with 100 μg dihydrotestosterone propionate and 30 μg diethylstilbestrol in 0.5 ml safflower oil or vehicle. As adults (P60) gerbils were castrated as necessary and implanted with Silastic capsules of testosterone propionate (10 μg). Four weeks later gerbils received injections of 1 μl of cholera toxin-HRP (500-μg/ml-HRP) in each side of the BC. After one week gerbils were perfused and alternate sections were processed for visualization of CT-HRP (Mesulam, J Histochim Cytochem 26:206) or stained for Nissl substance.

Neonatally castrated males, steroid-treated females and vehicle-treated males had clearly visible BC muscles. Vehicle-treated females did not. Virtually all of the CT-HRP labelled motoneurons were located in lamina X above the central canal (213/214). Neonatally castrated males had fewer motoneurons than did treated females (30 ± 2). These data suggest that the BC-spinal cord system either prenatally, or as a single low dose postnatally is sufficient to masculinize the male spinal cord above the central canal. Supported by HD 22869 (TRA).

529.5

The dorsolateral nucleus (DLN) and spinal nucleus bulbocavernosus (SNB) of the lumbar spinal cord are sexually dimorphic. Males have more neurons in both nuclei and males have larger neurons in the SNB. The spinal cords of 16 male and 9 female offspring of rats which received 1 μg of the anti-androgen, flutamide, on days 11 through 21 of gestation and 16 male and 16 female controls were examined.

Flutamide males had fewer DLN neurons (X̄=101) than did control males (X̄=488), but flutamide did not different from control females (X̄=280). Flutamide males also had significantly fewer and smaller SNB neurons than did control males. Flutamide did not significantly alter the SNB of females. These results support the hypothesis that the sexually dimorphic characteristics of the DLN and SNB are androgen dependent.

529.6
DELAYED EXPRESSION OF CALCITONIN GENE RELATED PEPTIDE (CGRP) IN SNB MOTONEURONS. Nancy G. Forger, Lynn Hodges and S. Marc Bredieowe. Dept. Psychology, Univ. California, Berkeley, CA 94720. Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) innervate sexually dimorphic perineal muscles. The development of this neuromuscular system is androgen dependent and differs temporally from that in other motoneuronal pools: 1) the normal period of motoneuron death is later in the SNB, with significant loss occurring postnatally and 2) neuromuscular synapse elimination occurs later in SNB cells. CGRP expression has been correlated with the period of motoneuron death in chicks and has recently been shown to be androgen regulated in rat SNB motoneurons in adulthood (Popper & Micevych, '89). We therefore examined CGRP-like immunoreactivity (CGRP-LI) in motoneurons of the lumbar cord of rats from postnatal day 3 to adulthood. In accord with previous findings, many lateral horn motoneurons were clearly positive for CGRP-LI on day 3 and all subsequent ages examined. In contrast, no CGRP-LI was detected in SNB cells on day 3 and only faint staining was observed on days 9-10. By day 16 occasional heavily labeled cells were found in the SNB and the adult pattern of CGRP expression was present by day 33. CGRP expression therefore appears to be significantly delayed in SNB motoneurons, suggesting an association with the events of cell death and/or synapse elimination.

529.7

Gap junctions are considered to play an important role in metabolic and electrical coupling between neurons. We studied androgenic influence on the expression of the snba mRNA for gap junction protein in the androgen-sensitive motoneurons in the spinal nucleus of the bulbocavernosus (SNB) by using in situ hybridization. Adult male rats (N=10) were castrated and implanted with siltastic capsules containing testosterone (T) or nothing. Animals were sacrificed 4 weeks later. A complementary DNA specific for the snba mRNA for gap junction protein (comasnba 32, a gift from Dr. D.A. Goodenough) was applied to in situ hybridization on cryostat sections of the lumbar spinal cords. Autoradiographic signals of comasnba 32 mRNA were found to be localized on the somata and proximal dendrites of SNB motoneurons. The number of signals per motoneuron in castrates was significantly smaller than that in controls. The values in castrates given T was not significantly different from that in controls. These evidences may regulate and express the gap junction gene in SNB motoneurons. (Supported by grant from the Ministry of Education, Culture and Science of Japan.)

529.8

Since motoneurons in the spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens, we compared the bulbocavernosus (BC) reflex, recorded in BC motor nerve in response to electrical stimulation of the contralateral pudendal afferents, in intact (n=16), castrated (n=14) and testosterone-treated (n=12) male rats under urethane anesthesia. No group differences in reflex latency, sensory or motor conduction velocity, or central delay were observed. We also examined the effects of stimulation of the contralateral pudendal sensory or BC motor nerves on extracellular antidromic motoneuron responses evoked by stimulation of the BC motor nerve. Stimulation of the contralateral pudendal afferents 7-35 msec prior to stimulation of the BC motor nerve reduced the amplitude of averaged antidromic potentials recorded in the SNB nucleus, whereas stimulation of the BC motor nerve stimulation had no effect. The magnitude of this suppression was much greater in intact and testosterone-treated castrated males than in castrates, indicating that androgen modulates the excitability of SNB motoneurons or other neurons involved in the BC reflex. Supported by NIH grant HD15021.
529.9 ANDROGENS DIFFERENTIALLY AFFECT SYNAPTIC EFFICACY WITHIN MOTOR UNITS OF A SEXUALLY DIMORPHIC MUSCLE. D. Naggard-D’Avino and A. A. Herberg, Dep. of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

The flexor carpi radialis (FCR) is a sexually dimorphic forelimb muscle used by male frogs during mating. Preliminary data indicate that motor units (MUs) in the shoulder and elbow regions of the FCR are innervated by motor neurons in spinal segments 2 and 3, respectively. Studies comparing castrated males (C) with castrates given testosterone (CT) show that FCRs from CT males contract more slowly and have larger fiber cross sectional areas (CSAs). The effects of testosterone on CSA and function are more pronounced in shoulder MUs. Using intracellular recording, we found that many shoulder fibers in CT muscles are innervated by subthreshold junctions. Only 63% of shoulder junctions in CT muscles triggered fiber action potentials with a single nerve stimulus compared to 97% of shoulder junctions in C muscles. Elbow junctions were found to be mainly suprathreshold in both CT and C muscles (80 and 98%). These results suggest that testosterone may preferentially affect the synaptic efficacy of neuromuscular junctions within motor units in the shoulder region. The larger CSAs of shoulder fibers from CT muscles suggest that lowered synaptic efficacy may result from a decrease in input impedance (R∞). Mean R∞ of shoulder fibers in CT muscles was significantly less than in C muscles (0.29 vs. 0.44 MΩ; p < 0.0002). We are currently investigating whether changes in neurotransmitter release may also contribute to altered synaptic efficacy.

Supported by NIH grant NS 27209.

529.10 CELLULAR SPECIFICITY OF STEROID INFLUENCES ON PROCESS OUTGROWTH OF IDENTIFIED MOTONEURONS IN CULTURE. L.L. Witten and R.B. Levine, ARL, Division of Neurobiology, U. Arizona, Tucson, AZ 85721.

Dramatic remodeling of the central nervous system (CNS) occurs during insect metamorphosis to accompany the changing lifestyle of the animal. In the moth, Manduca sexta, identified motoneurons exhibit cell-specific changes in morphological and biochemical properties during this transition and these changes are mediated by the steroid hormones, the ecdysteroids. To determine whether these changes result from the direct action of the steroid hormone on the neurons and to explore mechanisms underlying the cellular specificity of these effects, we have developed methods to culture identified motoneurons. We compared the effects of ecdysteroid on neurite outgrowth in two sets of identified motoneurons that have different developmental fates. Leg motoneurons display extensive growth of their central arborizations during adult development in vivo (Kent and Levine, '88) and have been shown previously to exhibit enhanced neurite outgrowth in the presence of ecdysteroids in vitro (Griffin and Levine, '89). In contrast to the leg motoneurons, the motoneurons that innervate the abdominal intersegmental muscles (ISM) do not exhibit significant dendritic growth during adult development in vivo, although they do alter the expression of a neuropeptide (Witten and Truman, '90). Accordingly, the ISM motoneurons do not show a steroid-mediated enhancement of neurite outgrowth in culture. Thus, these experiments confirm the specificity of steroid hormone action on process outgrowth.

530.1 ROLE OF THYROID HORMONES ON OLFACTORY NEUROGENESIS IN THE POSTNATAL RAT: A QUANTITATIVE 3H-THYMIDINE STUDY. M. Paternostro and E. Nelsemi Physiol. Dept., Univ. of Illinois, Urbana, IL 61801.

We have previously shown that the marked increase in surface area and total neuron number occurring in the olfactory epithelium (OE) of growing rats is severely reduced in rats made hypothyroid (HT) from birth, while rats allowed to recover from hypothyroidism at 25d showed complete recovery in these parameters by 90d. To better understand these effects, a quantitative 3H-thymidine study was undertaken. Normal (N) and HT rats were injected with 3H-thymidine (2μCi/g mouse) at 25d and 75d, while recovery rats were injected at 75d. Rats were sacrificed at 1, 5 or 15d post injection. Autoradiographs from septal OE were used to study the density of labeled cells in both the basal cell zone (bcz) and the receptor zone (rz). Results from the 25d injection group revealed at 1d pi, a 25% reduction in the density of labeled cells in the bcz of HT rats (p=0.003). In both groups at 5d and 15d pi, the density of labeled cells was markedly decreased in the bcz and proportionally the decrease in labeled cells in the rz at 15d pi was not significantly different between the two groups. Thus early thyroid deficiency reduces the rate of basal cell proliferation, but does not seem to significantly affect the survival and migration of the developing neurons. Work is currently being carried out on the 75d injection group.


In mature animals, thyroid hormone regulates cardiac β-adrenergic receptor numbers and the efficiency of receptor transduction. Exposure to excess thyroid hormone during early neonatal periods promotes the development of post-receptor transduction components independently from alterations in receptors; the current study determined whether normal development of β-adrenergic transduction requires endogenous thyroid hormone. To achieve neonatal hypothyroidism, pregnant rats were given propylthiouracil 20 mg/kg, s.c. beginning at gestational day 17 through postnatal day 5 and their offspring were given the same dose on postnatal days 1 through 5. We evaluated receptor binding capabilities and adenylyl cyclase activity in membrane preparations. Hypothyroidism produced an initial inhibition of [125Iioodo] binding to cardiac membranes, followed by a return to control levels after discontinuing treatment. During the first two weeks, isoproterenol-stimulated adenylyl cyclase activity displayed subsensitivity, corresponding to the deficits in β-receptor binding. Basal and forskolin-stimulated cyclase activities were initially unaffected, implicating a specific role for thyroid hormone in early stages of coupling of enzymatic responses to β-receptors. Thyroid induced deficiencies in basal and forskolin-stimulated adenylyl cyclase activity eventually emerged, suggesting that, at later stages, normal development of adenylyl cyclase requires prior establishment of a euthyroid state.

HORMONES AND DEVELOPMENT: THYROID HORMONE


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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
Recent evidence suggests various extracellular stimuli, such as growth factors and hormones, induce the cellular immediate early, or early response genes (ERGs) in neural cells. As such, ERGs may participate in the signal transduction processes that coordinate CNS development. However, whether the ERG products confer the intracellular specificity of the extracellular signals is not clear.

In this study, we examined the induction of ERG mRNA transcripts in insulin, hydrocortisone, and thyroid hormone in glial cells. We asked whether ERG expression occurs at intracellular specificities by comparing the patterns of ERG induction by these ligands individually or in combination. RNA transcribed from treated astrocytes or C6 gloma cells were examined by northern analysis using 32P-labeled cDNA encoding for c-fos, c-jun, junB, mgf, NFIb, or TIS11. Results showed that insulin, hydrocortisone, and thyroid hormone induced ERGs. The amounts and kinetics of ERG induction, however, differed in each treatment. Differences in the levels and time courses of mRNA expression were also observed when cells were treated with combinations of the ligands. Analysis of the ERG induction kinetics by various treatments showed that a specific pattern of ERG expression exists for a specific extracellular stimulus. These results suggest specific phenotypic responses to insulin, hydrocortisone, and thyroid hormone could be conferred by differential patterns of ERG expression. (Supported by NIH grant HD-00676 and DOE)


Both the short- and long-term behavioral consequences of early postnatal treatment with male and female Sprague-Dawley rats with 3,3',5-triiodothyronine (T3 - 0.5 μg body wt.) were examined using water maze and radial arm maze tests of navigational ability at different postnatal ages. In the dentate hippocampal field of PTU-treated rats compared to controls. In contrast, 3H-DAMGO binding was reduced to 25% of control in the deep cell region of the periventricular region of the hypothalamus. PTU treatment did not affect 3H-DAMGO binding in the amygdala, entorhinal cortex, lateral or medial geniculate nucleus or dorsal lateral geniculate nucleus. T4 supplementation reversed all of the PTU-induced mRNA binding alterations noted above. (Supported by AA00634, R01 HD20029 and CM00220)


The long-term behavioral consequences of neonatal treatment with 3,3',5-triiodothyronine (T3 - 0.5 μg body wt.) administered on postnatal days 1, 2, and 4 were examined by training adult male and female Sprague-Dawley rats on a discrete-trials peak-interval timing procedure to discriminate between visual signals paired with 20-sec and 40-sec intervals. Signals were presented in a double-stochastic manner and response rate functions were analyzed separately for each of the four trial types. Analysis of the discrimination index for peak functions showed that T3-treated rats were impaired in their ability to discriminate both within and between signal conditions relative to untreated males. In contrast, T3-treated females were improved in their ability to discriminate both within and between signal conditions relative to untreated females. No sex differences were apparent in control subjects. These results can be related to the sexual dimorphisms in basal forebrain and hippocampal cell morphology and the timing of T3 sensitivity of CA1 and CA3 pyramidal cells. Sexual dimorphisms and T3-induced cellular alterations are quantified using morphological and topological analyses (e.g., Sholl's method of concentric spheres) of 3D tracings of individual neurons obtained from Golgi-stained sections.

SYNAPTIC STRUCTURE AND FUNCTION II

1. A FAST ALGORITHM FOR SYNAPTIC CONNECTIVITY ESTIMATION. X. Yang(3), S.A. Shamma(3), and M.J. Bak(3).

The standard one-dimensional cable model of nerve cells assumes that their electrotonic structure do not change in response to synaptic input. This model is used in a great number of both theoretical and anatomical-physiological structure-function studies. Recently, we demonstrated that the membrane time constant \( \tau_m \) and the somatic input resistance \( R_{in} \) are used to characterize single cells. However, these studies do not take into account that neurons are embedded in a network of spontaneously active neurons. These networks may make up a large part of the membrane conductance, especially if the density of electrotonic membrane conductance, \( R_{in} = 100 \text{fF/cm}^2 \), is taken into account. We numerically simulated the electrical behavior of a morphologically identified layer V cortical pyramidal cell receiving input from 4000 excitatory and 1000 inhibitory cells firing spontaneously at 0-2 Hz. We find that over this range of neuronal background activity \( R_{in} \) can change by more than a factor 10 (7-80 ms, 18-153 MSf) and the electrotonic length of the cell by a factor 3. Thus the global activity of the network controls how individual cells perform spatial and temporal integration. This provides possible mechanisms for gain control, coincidence detection, and dynamic control of tuning curves.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
531.3 EFFECTS OF MICROWAVE ENVIRONMENT COMPOSITION ON CIRCUIT FUNCTION USING PUSH-PULL PERFUSION WITH MICROELECTRODE RECORDING IN THE CAT CATECHOLAMINES. W.R. Beggstedt and H.G. Gelfinger, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH 45435.

In neuroblastoma-anesthetized adult cats, interstitial cSF (i-CSF) was withdrawn (-6.6 kPa) and there was no change in arterial blood pressure (31). The Push-Pull Cannula (PPC) (previously described (11)) was placed onto the surface of the medial (cuneate nucleus and was not moved; perfusion rate was 0.5 mL min⁻¹). The PPC perfused was collected in 5-min periods without (a-CSF) and (b-CSF), dopamine and norepinephrine concentrations were determined. To assess circuit function after per fusion during periods of normal amplitude, slightly greater duration and lower frequency activity, rest, or exposure to substances known to affect synaptic transmission. The synaptic vesicle was exposed to a particular condition, then the synaptic vesicle was exposed to a particular condition, then


Tonic in vivo stimulation of a crustacean phasic motoneuron alters the transmitter releasing properties of the motor axons and may be more similar to those of a tonic motoneuron (J. Neurosci. 1.549-467, 1985). The activity-dependent synaptic changes include a long-term reduction in initial neurotransmitter release. We have recently observed that local application of the proximal region of a crayfish phasic motoneuron (abdominal motoneuron, F3) in vitro is sufficient to produce this reduction in transmitter release from motor terminals (J Neurosci. 16:1162, 1996). Local application of Ca⁺⁺ in the induction of this synaptic change, the proximal region of F3 was depolarized in Ca⁺⁺-free saline containing the Ca⁺⁺ channel blocker Ma+(6 mM). While conditioning in normal saline resulted in a significant 30% decrease in initial EPSP amplitude (p<.01, t-test; n=12), blocking Ca⁺⁺ influx during conditioning prevented this reduction in EPSP amplitude (p<.05, n=10). These results demonstrate that Ca⁺⁺ influx at the proximal region of a motoneuron can influence transmitter release from distant terminals.

To examine the role of protein synthesis, the protein synthesis inhibitor, cycloheximide (CHX), was bath applied during conditioning. CHX (0.6 mM) applied 2 hr prior to conditioning prevented the block of EPSP amplitude. However, CHX application at the onset of stimulation did not block the effect of the conditioned axon. This study suggests that a pool of preexisting proteins is essential for the induction of this activity-dependent change in initial transmitter release (J. Neurosci. 10:1099-1109, 1990). Supported by NSF grant BNS-8720135.

531.5 ENZYMATICALLY DISSOCIATED NMJs FUNCTION ALMOST NORMALLY AFTER MANUAL REATTACHMENT OF TERMINAL TO ENDPLATE. R.S. Wilkinson and S.D. Lunn,* Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

NMJs are precisely organized at the subsynaptic level, e.g., the close proximity of pre- and postsynaptic elements and the alignment of active zones opposite postsynaptic gutters. The functional importance of these features has been assumed but not directly tested. To address this we 'reconstructed' NMJs by manually positioning enzymatically-detached nerve terminals over synapses in the snake transversus abdominis muscle. Reconstructed NMJs functioned immediately (<0.1 s). Mepps were of normal amplitude, slightly greater duration and lower frequency than controls. FSPs evoked in low Ca⁺⁺ solution comprised fewer quanta than controls, but diminished mepp frequency and PSP amplitude in proportion to the involvement of potassium conductance changes. The M, receptor required for basic function, or, alternatively, such alignment occurs but diminished mepp frequency and PSP amplitude in proportion to the involvement of potassium conductance changes. The M, receptor required for basic function, or, alternatively, such alignment occurs but diminished mepp frequency and PSP amplitude in proportion to the involvement of potassium conductance changes. The M, receptor required for basic function, or, alternatively, such alignment occurs but diminished mepp frequency and PSP amplitude in proportion to the involvement of potassium conductance changes. The M, receptor required for basic function, or, alternatively, such alignment occurs but diminished mepp frequency and PSP amplitude in proportion to the involvement of potassium conductance changes. The M, receptor required for basic function, or, alternatively, such alignment occurs but diminished mepp frequency and PSP amplitude in proportion to the involvement of potassium conductance changes. The M, receptor required for basic function, or, alternatively, such alignment occurs...

The anticholinergic action of perezone is probably related to the spontaneous activity, it blocks acetylcholine receptors and probably decreases spontaneous acetylcholine release.

CALCIUM REGULATION IN RAT POSTERIOR PUYTARY NERVE TERMINALS. A.O. Johnson* & D.A. Defenere, Dept. of Biological Sciences, Columbia Univ, NY NY 10021.

The posterior pituitary gland is composed of neurones and their terminals. This makes the gland a unique preparation for the study of synaptic mechanisms, specifically calcium regulatory mechanisms. We have used intact posterior pituitary gland slices to study calcioresponses (80-100 μM) incubated with the membrane permeant calcium indicator dye, fluo-3/AM, in order to characterise the calcium regulatory mechanisms in the terminals. Data from standard fluorescent microscopy in single or stained tissue is complicated by signals from surrounding structures. By using a confocal microscope we have been able to circumvent this problem. The initial phase in this project involved dealing with these technical problems. The first was to determine a range of laser intensities which gave reproducible signals without significant bleaching during experiments that lasted over forty minutes. Second, the application of confocalogues during initial experiments caused tissue movements which interfered with data collection. We overcame this problem in the initial preparation by tacking the tissue down with minute pins. The third was the calibration of changes in fluo-3 fluorescence with changes in intracellular calcium concentrations. This included the determination of whether the lasers were stable over time and the photobleaching was significantly over time. We have used the non-fluorescent calcium ionophore, 4-bromo-2,9-dimethoxy-AC19, to determine the range of fluorescent responses to changes in the concentration of extracellular calcium and the accessibility of the dye. Data from entocrine cells and neural soma suggest that there are two major sources of calcium: extracellular calcium entering through calcium released from intracellular stores. Posterior pituitary nerve terminals possess voltage gated calcium channels, however, the presence of releasable intracellular stores remains open. We are currently examining this question as well as the effects of neurotransmitters on calcium regulation in these nerve terminals.

SYNAPTIC ULTRASTRUCTURE OF TRIGEMINAL MOTOR NEURONS IN CATS. N. Capraro, V. Hubbard*, and J. Bernardone*, Dept. of Physiol., Univ. of MD, Baltimore, MD 21201, Dept. of Anatomy Univ. of MS, Jackson, MS 32916.

This study compares the synaptic ultrastructure along the soma and proximal dendrites of masticatory and digastic motor neurons in cats. Horseradish peroxidase (HRP) injections were made into the digastic muscle in 2 cats and into the masticatory muscle in 2 cats. Vibratome sections were reacted with diaminobenzidine to identify cells containing retrogradely transported HRP. Labeled neurons were trimmed from the sections, osmicated, processed into plastic, and prepared for ultrastructural study. Ten masticatory and 10 digastic neurons, sectioned through the nucleolus, were studied. Terminal boutons in both cell populations contained either small clear vesicles, a mixture of clear vesicles and large dense-core vesicles, (LDCV), or small vesicles flattened. Terminals containing flattened vesicles were more numerous along digastic neurons while clear vesicles were more common in masticatory neurons. Synaptic density along digastic somata was greater than along masticatory soma (38% vs. 28%). In both populations, the largest terminals were associated with extensive subsynaptic cisterns and postsynaptic accumulations of granular endoplasmic reticulum. Some terminals were identified as asymmetric contacts. Occasionally, boutons along the soma and proximal dendrites of masticatory neurons were associated with prominent subsynaptic bars. In spinal cord preparations, these may be important for the formation of excitatory and inhibitory postsynaptic potentials.

ACCOMPANYING THE PERIPHERAL MOTOR AXONS IN MANDUCA LARVAE. M.B. Rheuben and D.M. Autio*, Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Insects have elaborate neurosecretory systems whose products are involved in development, body size determination, and function of muscle. Nerve branches innervating skeletal muscle in Manduca include small axons of a neurosecretory (ns) nature and possibly a functional specialisation. We have examined the ultrastructure of these ns axons in the vicinity of the neuromuscular junctions using serial sections. One ns axon accompanies a single motor axon to each muscle fiber. The ns axon is located over the outermost glial processes investing the motor axon, may at times be directly exposed to the hemolymph, and forms varicosities ranging from 0.24-1.52 μm in diameter and 0.4-6.7 μm in length, and with inter-varicosity distances averaging 5.0 μm. Within varicosities, dense- and clear-filled vesicles clustered in an apparent pre- and postsynaptic "specialisation", many of which are in direct apposition to an ensheathing glial cell process. The ns axon itself, though close, does not appear to come into direct contact with the muscle fiber nor to enter into the neuromuscular junction. The results suggest that the ns axon could have a "distant" neuromodulatory influence on the neuromuscular junction or the muscle fiber, but also that it might interact in some direct way with the glial sheath.

FLUCTUATION ANALYSIS OF SINGLE FIBER EPSPS IN THE SPINAL CORD OF THE BULLFROG. Ch. Strieker and H.-R. Lüscher, Dept. of Physiol., Univ. of Bern, Switzerland.

Synaptic transmission from muscle afferents to motoneurons in the frog spinal cord is both electrical and chemical. By comparing the fluctuations of the electrically and chemically evoked potentials, it should be possible to determine whether fluctuations are a result of failure to release transmitter or of uncertainty of potential propagation in axons. In addition, it is expected that the method of variance analysis is not sufficiently sensitive to detect an expected variance of the electronic component because of contaminating noise. Therefore, we used a different approach for comparison of labeled terminals. We have measured single fiber EPSPs in motoneurons elicited by tapping the toe limb of a frog muscle (1-5 Hz). The animals were anesthetized with ice and the spinal cord and muscle rapidly dissected. The spinal cords were sectioned along the mid line and the preparation superfused with oxygenated Ringer (14°C). Motoneurons of the triceps muscle were impaled with microelectrodes (2-4 nK-citr., 30-70 Ω) and identified by antidromic stimulation. EPSP and noise measurements of both those cells were included in the analysis if the membrane potential during recording was < -55 mV and did not vary more than 5 mV. Afferent action potentials of the triceps nerve were used for spike triggered averaging of the EPSPs and noise records. The components of the EPSP were identified by shape and latency. The average and variance time courses of the EPSP were calculated and the latter corrected for noise variance. Amplitudes of the EPSPs were then grouped into two classes differing by at least two standard deviations of the noise. EPSPs belonging to the same amplitude class were averaged again and the results analyzed for shape and latency. The electronic component of the EPSP in the small amplitude group is often smaller in size than in the large amplitude group and that the quotient of electronic to chemical amplitude is always greater in the smaller group. The results suggest that the probability of transmitter release at this synapse is determined by two factors: impulse propagation and release process.

Quantal analysis can reveal important aspects of synaptic transmission. However, it is difficult to do quantal analysis in CNS neurons due to the sporadic release and low understanding of quantal variance. To solve these problems, we obtained whole cell patch clamp clamp currents in the chick ciliary ganglion (CG) in situ. We observed a quantal release of excitatory amino acid receptors. The experimentally observed quantal variance amplitude appeared largely due to statistical properties of the channels. Therefore, we applied non-stationary noise analysis to each group of quantal events to determine single channel conductance, number of channels, and probability of channel opening under endogenous EPSCs associated with synaptic drive to parasympathetic motoneurons. We found the smallest quantal synaptic current was due to activation of ~20 channels with single channel conductance ~30 pS. In summary, these results represent a novel approach for quantal analysis in CNS neurons and new insights into quantal release mechanisms in situ. Supported by NIH Grant NS 24742.

532.2 THREE POTASSIUM CHANNELS IN RAT NERVE ENDINGS. K. Bielefeld, J.L. Rotter, M.B. Jackson. Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

Voltage-dependent K channels determine the shape and duration of action potentials. Presynaptically, they influence transmitter release by limiting depolarization and, thereby, Ca influx through voltage-sensitive Ca channels. We used single channel techniques to study the K channels in cell-attached patches of nerve endings from rat posterior pituitary slices. In nominally symmetrical potassium, 3 K channels could be distinguished on the basis of channel conductance and kinetics. The most frequently observed channel had a conductance of 21 pS, and inactivated rapidly (tau: 18 ms). A second K channel had a larger conductance of 110 pS, and inactivated more slowly (tau: 82 ms). A third K channel with a slope conductance of 16 pS activated slowly and did not inactivate during a 300 ms depolarization. An excellent correspondence was exhibited between the properties of these three K channels and the various components of macroscopic K current recorded in tight-seal whole-terminal experiments. Thus, the biphasic inactivation kinetics and complex voltage dependence of activation in macroscopic current can be explained in terms of the underlying behavior of three different K channels. The importance of voltage dependent K current inactivation in action potential broadening and frequency dependent facilitation suggests a physiological role for these K channels in synaptic plasticity.

532.3 CALCIUM CURRENTS RECORDED FROM PRESYNAPTIC TERMINALS IN THE CHICK CILIARY GANGLION. H. Yawo and A. Momiyama*. Department of Physiology, Kyoto University Faculty of Medicine, Kyoto 606, Japan.

The properties of calcium currents of vertebrate presynaptic nerve terminals were studied under whole-cell voltage clamp in large calyciform terminals of the chick ciliary ganglion identified by fluorescence, dextran-tetramethylrhodamine applied to the cut end of the oculomotor nerve 5 hrs prior to the experiment. When whole-cell recordings were made simultaneously from both the presynaptic terminal and postsynaptic ganglion cell, an EPSP (or EPSC) could be elicited by activation of presynaptic calcium currents. There was no evidence for the existence of low-threshold calcium currents in the presynaptic terminal. The high-threshold calcium current was potentiated by a dihydro­pyridine agonist, Bay K 8644. The potentiated calcium current as well as the background calcium current was irreversibly suppressed by ω-conotoxin GIVA (ω-CgTX). However, 20-50 % of the control calcium current were resistant to 10 μM-ω-CgTX. The ω-CgTX-resistant component was not sensitive to Bay K 8644.

These results suggest that in the chick ciliary calyx, there are at least 2 components of high-threshold calcium currents: the one component is sensitive to both Bay K 8644 and ω-CgTX, and the other is insensitive to both.

532.4 ALTERATION IN MICROFILAMENT ORGANIZATION OF BOVINE ADRENAL CHROMAFFIN CELLS DURING MASTOПARAN-EVOKED EXOCYTOSIS. K. Kumakura1*, T.Sakurai2*, M.Iizumii1* and Y.Nomura2*. 1Life Science Ins., Sophia Univ. and 2Dept. Pharmacol., Univ. of Tokyo, Japan.

Mastoparan, a tetradecapeptide from wasp venom, is known as a non-specific secretagogue for a variety of cells. Using a real-time monitoring technique, we found that the peristaltic stimulation of bovine adrenal chromaffin cells with mastoparan evoked the transient secretory response with a biphasic dose-response curve. In addition, the stimulation of chromaffin cells with 3-7 μM mastoparan, continuous release of catecholamines with a constant magnitude was evoked till withdrawal of mastoparan. These secretagogue action of mastoparan was completely independent of Ca2+. By use of FITC-phalloidin and immunofluorescence microscopy, we revealed that subplasmalemmal microfilament network was disrupted during mastoparan-evoked exocytosis either in the presence or absence of Ca2+. Such disassembly of microfilament network was also observed during nicotine-evoked exocytosis. These results suggest that mastoparan is a useful tool to study cellular mechanism for exocytosis.

532.5 INCREASE OF EXTRACELLULAR K+ TO 10-12 mM ABOLISHES EXCITATORY SYNAPTIC TRANSMISSION BETWEEN CEREBELLAR NEURONS IN TISSUE CULTURE. W. Rabbe. Neurology, VA Med. Ctr., Dept. Neurology, Physiology and Neuroscience Graduate Program, University of Minnesota, Minneapolis, MN 55417.

Increased extracellular K+ (K+) has been reported to affect axonal conduction and synaptic transmission by inactivating voltage-dependent K currents in the presynaptic terminal. The high-threshold calcium current was irreversibly suppressed by ω-conotoxin GIVA (ω-CgTX). However, 20-50 % of the control calcium current were resistant to 10 μM-ω-CgTX. The ω-CgTX-resistant component was not sensitive to Bay K 8644.

These results suggest that in the chick ciliary calyx, there are at least 2 components of high-threshold calcium currents: the one component is sensitive to both Bay K 8644 and ω-CgTX, and the other is insensitive to both.


NH4+ (5 mM) produces a conductive block in presynaptic terminals and interrupts synaptic transmission (J Neurophysiol 52:1418, 1989). In the presence of NH4+, the peak amplitude of the EPSCs is reduced by ~50% due to the action of NH4+ and K+ on synaptic transmission. Whole cell patch voltage clamp recordings were obtained from large (>20 μm) cerebellar neurons, presumably Purkinje cells, grown in primary dissociated tissue culture. A unipolar patch pipette was used to stimulate nearby cells (<10 μm), presumably granule cells, to evoke monosynaptic EPSCs. Normal extracellular solution contained 5 mM K+ and 120 mM Na+. Increases of K+ to 10 mM reversibly abolished the evoked EPSCs in most cells. In those cells, in which 10 mM K+ did not abolish the EPSC, 12 mM K+ abolished the EPSC. With increased K+ abolished the evoked EPSCs, neurons were still able to generate action currents and to produce spontaneous TTX-independent miniature EPSCs. The abolition of the evoked EPSC was always preceded by a transient increase in the EPSC amplitude.

The abolition of excitatory synaptic transmission between cerebellar neurons by K+ 10-12 mM is consistent with a depolarizing block for action currents, spontaneous in synaptic terminals. This suggests that for the study of transmitter release from neurons it may be best to increase K+ to 8-9 mM to obtain increased spontaneous synaptic activity without blocking depolarization of presynaptic terminals. In this case, it can be suggested that in those seizures which increase K+ to 10-12 mM, increased K+ may abolish neuronal excitatory synaptic transmission and may contribute to seizure termination.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991

THURSDAY PM   1324 PRESTNAPTIC MECHANISMS
FREQUENCY-MODULATION OF SYNAPTIC EFFICACY IN EMBRYONIC RAT SPINAL CORD SLICE CULTURE. Ch. Lüchinger, J. Streit, H.P. Claman and H.R. Lüchinger. Department of Physiology, University of Berne, Switzerland

Frequency-dependent alterations of synaptic efficacy are believed to functionally demarcate stages in the development of synapses. We investigated the effects of frequency fluctuations in a palmate-like network of spinal cord neurons in organotypic slice cultures. EPSPs and IPSPs were recorded from motoneurons following stimulation of different frequencies of DRG-cells, using conventional intracellular electrodes. A depression in amplitude by up to 100% of EPSPs and IPSPs and an increase in latency by roughly 1 ms was found, when the frequency was increased by the factor 10 (range 0.1-10Hz). The decrease in amplitude showed the same quadratic steps as were seen with amplitude fluctuations at constant frequency of stimulation. In addition, steps of multiple quanta were consistently found, suggesting a propagation failure at a higher order in the arborisation, leading to quantal switches off in groups. Reducing extracellular Ca++ concentration from 5mM to 1mM shifted the frequency-dependence of these effects towards higher frequencies. When action potentials invading DRG somata after stimulation of their axons were investigated using the same frequency protocols, an increase in latency (17-35%) and its variance as well as the appearance of partial and complete failures were observed. These effects were again less pronounced with a reduced Ca^{2+}-content of the perfusion solution. From these findings it is concluded that synaptic depression is of presynaptic origin, and that there is strong evidence for a conduction failure in arborisation as a underlying mechanism.

GABA VIA PRESYNAPTIC GABA_{A} RECEPTORS INHIBITS EXCITATORY POSTSYNAPTIC POTENTIALS IN NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS. S.Y. Wu, S.L. Dun*, C. Ren, N.J. Dun and A.G. Karczmar, Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43669 and Hines VA Hospital, Hines, IL 60153.

Nerve fibers and a few small diameter cells immunoreactive to GABA antiserum were observed near the lateral horn of the rat spinal cord. The effects of GABA and baclofen on the synaptic activity of sympathetic preganglionic neurons (SPNs) in neonate rat thoracic spinal cord slices were evaluated with intracellular recording techniques. Superfusion of GABA (25-100 μM) and (-)baclofen (1-10 μM) consistently depressed the excitatory post-synaptic potentials (EPSPs) evoked by action potentials, without causing a significant change of membrane potential or input resistance of the SPNs nor the depolarizations induced by pressure applications of glutamate. GABA and baclofen also eliminated spontaneously occurring EPSPs. The synaptic depression of GABA and baclofen antagonized by the GABA_{A} receptor antagonist 2-OH-saclofen (20 μM) but not by the GABA_{B} receptor antagonist bioculin (10-50 μM). These results suggest that GABA may be involved in the regulation of transmitter release by acting on presynaptic GABA_{A} receptors in the lateral horn. (Supported by NS18710)

A NOVEL EFFECT OF OPIODS ON SYNAPTIC INHIBITION. V.A. Doze, G.A. Cohen and D.V. Madison. Department of Molecular Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305.

Opioid receptor agonists, such as the stable enkephalin analog, D-Ala^{2}-MePhe^{4}-enkephalinamide (DAM), potently reduce synaptic inhibition of pyramidal neurons in the dentate gyrus by a decrease in the evoked inhibitory postsynaptic potential (IPSP). Although DAMA has been shown to directly hyperpolarize and reduce the action potential charge of interneurons, it is not known whether these effects are via their action on presynaptic terminals. We investigated this by monitoring the effects of DAM on spontaneous IPSPs (sIPSPs) and their frequency at various test frequencies. Under whole-cell conditions (30°C), using intracellular recordings and various pharmacological probes, we examined the effect of DAM on: 1) sIPSPs evoked by IPSPs, 2) spontaneous evoked IPSPs, 3) spontaneous evoked IPSPs (in the presence of pyridine N-oxides) to enhance monosynaptic transmission), 4) micro iontophoresis of Damino acid (DA) or GABA (GABA) in the stratum oriens, and CNQX/APV in the extracellular ACSF), and 4) micro iontophoresis of GABA (GABA). The type II pyrethroids, deltamethrin (Emax 25 mV depol.; E80 10-5 M), cypermethrin (Emax 35 mV depol.; E80 10-5 M) and fenvalerate (Emax 17 mV depol.; E80 10-5 M) elicited concentration-dependent, tetrodotoxin (TTX)-sensitive plasma membrane depolarization of about 5-10 mV presynaptically in the GABAergic terminal. These pyrethroids also elicited TTX-sensitive release of HAC. These data suggest that the synaptic location of the opioid disinhibition. In 5 preparations elevation of [Na+]i during a tetanus, by blocking the Na/K pump with ouabain, increased the posttetanic time constant for decay of [Na+]i from 6.5 ± 5.2 min in normal to 15.7 ± 14 min in high [Na^{+}]i (532.9). Supported by NIH grants ES05006 and ES01985.

THURSDAY PM

PRESYNAPTIC MECHANISMS

532.4 EFFECTS OF PYRETHROID INSECTICIDES ON SYNAPTIC FUNCTION. J.T. Ecles, J.M. Propp* and P.A. Holman*. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

The neuroexcitatory actions of two toxicologically distinct classes of pyrethroid insecticides and deltamethrin were characterized in rat brain synaptosomes using 3H-tetraphenylphosphonium to measure membrane potential and measuring the release of 3H-5-acetycholine ([H]-ACh). The type II pyrethroids, deltamethrin (Emax 25 mV depol.; E80 10-5 M), cypermethrin (Emax 35 mV depol.; E80 10-5 M) and fenvalerate (Emax 17 mV depol.; E80 10-5 M) elicited concentration-dependent, tetrodotoxin (TTX)-sensitive plasma membrane depolarization of about 5-10 mV presynaptically in the GABAergic terminal. These pyrethroids also elicited TTX-sensitive release of [H]-ACh. These data suggest that the synaptic location of the opioid disinhibition.
532.13 KINETICS OF Na/Ca EXCHANGE IN RAT BRAIN PRESYNAPTIC TERMINALS. G. Fontana & M. P. Blaustein. Dept. of Pharmacol., Univ. of Maryland Med. Sch., Baltimore, MD 21201

"Ca" fluxes were measured in rat forebrain synaptosomes in the presence and absence of external Na+, Na0. To determine "initial rates" and some kinetic parameters of Na/Ca exchange, 1 to 3 sec incubations were used; [3H]Naphthol was applied for 3 ± 1 sec (Ca1/2 uptake = 0.2 mM, but declined after 1 sec at [Ca1/2] = 0.5 mM. Virtually all the "Ca" uptake activated by removal of Na0 (Δ[Ca]) was internal Na" (Na0 dependent). Δ[Ca] was measured as a function of [Ca1/2] at 1 sec, at 37°C: apparent K1/2 = 0.25 µM; Δ[Ca] = -1200-1400 pmol/mg protein x sec (picogram/µmole); Hill coefficient (nH) = -1. The Na0-dependent "Ca" uptake ([Ca0] = 0.2 mM) was inhibited by Na0 with an apparent K0 = 50 mM and n0 = -2.6 (1 sec incubation, 37°C). Δ[Ca] = Na0-dependent "Ca" uptake was observed in the apparent absence of external and internal K+, however, raising (K+, from nominal "0" to 5 mM increased the Na0-dependent uptake in a concentration-dependent manner. After "Ca" loading (+ 6 nmol total Ca/mg protein, almost all the "Ca" efflux was Na0-dependent (Na0/Ca exchange): -1100 pmoles/mg without FCCP, -1600 pmoles/mg with FCCP (to release "Ca" from mitochondria and raise [Ca+]i). Thus, maximal exchanger-mediated "Ca" flux rates in both directions were about 1200-1400 pmoles/mg at 37°C. With this very large capacity (Ja/Δ[Ca]), the exchanger in nerve endings may play a major role in regulating [Ca+]i, and may even participate in the termination of transmitter release by rapidly extruding "Ca".


Ion flux measurements were performed in a large-capacity (high maximal flux) Na/Ca exchanger in vertebrate nerve terminals. We used polyclonal antibodies raised against dog heart sarcolemmal Na/Ca exchanger to localize the exchanger in immunofluorescence preparations by immunofluorescence microscopy. This antibody recognizes the major polypeptide of the Na/Ca exchanger (MW = 160 Kd) in immunoblots of dog heart and rat skeletal muscle and brain. In frozen sections through the immunobluection junction of rat skeletal muscle, the antibody did not precipitate the Na/Ca exchanger, labelled functional regions more intensely than nearby extrajunctional areas. In fixed and detergent-permeabilized Xenopus nerve and muscle co-cultures, the antibody labelled the nerve in a punctate fashion, suggesting that the exchanger is distributed heterogeneously. Labelling was observed where the nerve traversed the muscle as well as in nearby regions of axons not in contact with muscle. At nms where the nerve had made a turn around the muscle, the labelling remained associated with the nerve. This labelling was not detected with preimmune serum. Specific labelling of muscle could not be detected. We conclude that the Na/Ca exchanger is present at high concentration in pertinent regions of the nms, where it likely contributes to Ca homeostasis.

532.15 REDUCTION OF SLOW CHOLINERGIC EPSPS IN THE HIPPOCAMPUS OF AGED RATS. L. Taylor and W. H. Griffith. Dept. Med. Pharmacol. & Toxicol., College of Medicine, Texas A&M University, College Station, TX 77843.

Central cholinergic transmission has been proposed to decline as a function of age. We have tested this directly using cholinergic synaptic transmission in the in vitro rat hippocampus. Intracellular recordings were made from CA1 pyramidal cells using electrodes containing 2 M K-methyl sulfoxide. Slow excitatory post-synaptic potentials (slow-EPSPs) were evoked by repetitive stimulation (20 Hz, 500 ms duration) of the stratum oriens in the presence of physostigmine (1µM). Reproducibility was defined as 4-6 identical trains ± 18% or less. At 8, 10 and 20 months, the amplitude of the slow-EPSPs was reduced in 18-23 mo (Supported by NIH Grants RR05814 and AG07805).

532.16 PHARMACOLOGICAL PROFILE OF THE SELECTIVE D2 Dopamine Agonist, N-0923. D. S. Friedman, D. K. Krause and S. P. Duckles. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92715.

We have previously shown in the rat tail artery that N-0923 activates prejunctional D2 receptors to inhibit norepinephrine release. However, concentration-response curves were complex, such that at concentrations of 10-7 M N-0923 no longer inhibited norepinephrine release. When α2 adrenoceptors were blocked with yohimbine (10-6 M), concentration-response curves for N-0923 became monophasic up to a concentration of 10-7 M. N-0923 (10-7 M) inhibited contractile responses with an EC50 of 5.2 x 10-9 M and a maximum inhibition of 68 ± 2% and inhibited [3H]epinephrine influx with an EC50 of 8.6 x 10-9 M and a maximum inhibition of 72 ± 3%. Inhibition of both contractile responses and [3H]epinephrine influx was blocked by sulpiride confirming a D2 receptor site of action. At a concentration of 10-6 M N-0923 increased baseline contractions as well as [3H]epinephrine influx in tissues prelabeled with [3H]norepinephrine, effects which were unchanged in the presence of yohimbine or sulpiride. N-0923, an enantiomer with less potency at D2 receptors, was equipotent at increasing baseline [3H]epinephrine influx indicating a lack of stereoselectivity. These findings suggest that N-0923, at concentrations of 10-7 M or higher, blocks α2 adrenoceptors causing an increase in adrenergic transmitter release. Given the lipophilic nature of these compounds, the increase in baseline [3H]epinephrine influx produced by 10-6 M N-0923 or N-0924 may reflect a t-2 anti-neuronal action. Supported by NIH fellowship MH09902.

532.17 SENSITIVITY OF PREJUNCTIONAL α2 Adrenoceptors TO THE AGONIST UK14304 DECLINES WITH AGE IN THE RAT TAIL ARTERY. J. Buchholz and S. P. Duckles. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

Our previous studies have shown that fractional norepinephrine release from adrenergic nerves in the F-344 rat tail artery significantly increases with age. Furthermore, the sensitivity of prejunctional α2 adrenoceptors to the antagonists yohimbine or idazoxan also declines with age. We have complemented these studies with the use of the selective α2 agonist UK14304. This agonist inhibits fractional norepinephrine release as measured by HPLC and electrochemical detection. Our data (Table 1) show that there is no age-related difference in the inhibitory effect of UK14304 on fractional norepinephrine release at each concentration. These findings suggest that the function of inhibitory prejunctional α2 adrenoceptors is significantly altered with age in the rat tail artery. This may be an important variable in explaining the age-related increase in stimulation evoked norepinephrine release previously reported.

<table>
<thead>
<tr>
<th>Age in Months</th>
<th>1-5</th>
<th>5-6</th>
<th>6-12</th>
<th>12-18</th>
<th>18-24</th>
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<tbody>
<tr>
<td>% Inhibition of Fractional NE Release by UK14304</td>
<td>12.9</td>
<td>18.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
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* Significantly different from 20 months, P<0.01.

Supported by NIH AG08912 and AG05498.

532.18 MULTIPLE TARGETS OF ARACHIDONIC ACID IN THE MODULATION OF SYNAPTIC TRANSMISSION. J. E. Richardson, F. Bahl, W. L. Simp and P. G. Haydon, Dept. of Zoology and Genetics, Iowa State University, Ames, IA, 50011.

FMRFamide causes a presynaptic inhibition of synaptic transmission by neuron B5 of Helixoma trivittata. This modulation is due to a coordinate reduction in the calcium current and in the response of the secretory machinery to internal calcium. By voltage-clamping acutely dissociated spherical neurons of B5, we now show that FMRFamide (threshold concentration 3x10^-7 M) also increases an outward current. This current reverses around -85 to -90 mV, shows little rectification and shifts by 40-50 mV with a 10% increase in external K+, consistent with it being a voltage-independent K+ current.

We have investigated the role of the arachidonic acid (AA) cascade in mediating some of these modulatory actions. Bath application of AA (10μM) increases an outward current that has properties similar to the FMRFamide and K+ current. Furthermore, addition of the phospholipase-A2 inhibitor BPB (10μM) or the lipoygenase and cyclooxygenase blocker NDGA (10μM) reduces FMRFamide activation of the outward current.

Somata of neurons B5 and B19 form a giant cholinergic synapse under appropriate culture conditions. We have developed a technique for producing long-lasting synchronous release of FMRFamide in the presence of inhibitory post-synaptic currents (MIPSCs) at this synapse by elevating calcium levels through the micro-injection of the calcium cage DM-nitrophen. Under these conditions both FMRFamide and AA (10 and 50μM) cause a reduction in the frequency of MIPSCs in the postsynaptic neuron B19. Addition of AA (but not FMRFamide) also reduces the magnitude of MIPSCs. The effects of BPB and NDGA on the inhibition of secretion by FMRFamide are currently in progress. Takeda together these data suggest that the AA cascade mediates some elements of synaptic inhibition produced by FMRFamide.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
SYNAPTIC TRANSMISSION IS ALTERED IN ADULT SHR RATS.


Exaggerated sympathetic nervous activity, due to an increased neuronal excitability, has been implicated in both the development and maintenance of hypertension in the spontaneously hypertensive rat (SHR). However, transmission of high frequency stimuli through the superior cervical ganglion (SCG) of adult SHR is diminished. This suggests that synaptic transmission is depressed during the development of hypertension in SHR. In order to test this various parameters of synaptic transmission were assessed using in vitro isolated SCG from SHR, WKY and Wistar rats. Premotoric stimulation was achieved using a suction electrode on the cervical trunk and fast EPSPs were recorded using standard intracellular techniques. Use of the variance method determined that quantal content was increased, but quantal size was the same in SHR neurons when compared to those from WKY. Short term facilitation, determined from paired stimuli with interpulse intervals of 25-1000 ms, was found to be smaller in magnitude and shorter in duration in SHR neurons compared to WKY neurons. Trains of EPSPs were observed to facilitate less and depress more in SHR neurons when compared to WKY neurons. In low Ca2+ high Mg solution these differences were reduced, but not removed. The small amount of depression observed in SHR neurons during paired stimulation in normal solution was eliminated by the low Ca2+ high Mg solution. In addition, under these conditions trains of presynaptic stimuli gave rise to EPSPs in SHR and WKY neurons which increased in size throughout. These observations will be discussed in terms of the relationship of sympathetic nerve activity and hypertension. Supported by PHS grant HL 43636.

INTRACELLULAR Ca2+ MEASUREMENTS WITH FURA-2 IN PRESYNAPTIC TERMINALS IN BULLFROG SYMPATHETIC GANGLIA.

V. Y. Peng and R. S. Zucker. Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720.

To investigate the Ca2+ dependence of neurotransmitter release in bullfrog sympathetic ganglia neurons, where the preganglionic C fibers release both ACh and LHRH, a method was developed to fill the presynaptic terminal boutons with Fura-2. Fura-2 appears to be transported to the terminal boutons via a fast method. Typically, at least 30 surface cells possessed filled boutons. For a given neuron, up to 50 individual boutons were observed. Fura-2 concentration in boutons was 100-500 μM. Ca2+ elevation in synaptic boutons due to a single stimulus to the presynaptic fiber can be detected by a photomultiplier. Most boutons had a resting Ca2+ concentration of ~30 nM. Only when the resting Ca2+ was ~70 nM, was there spontaneous release of ACh, which was measured as an increase in Ca2+ concentration of ~50 nM. The effect of ACh and LHRH on Ca2+ concentration was determined. For boutons on C neurons, the LHRH receptor antagonist (D-Phe1, D-Pro2, D-Trp4, 6)-LH-RH, 20 μM) decreased the Ca2+ elevation evoked by stimulation to the preganglionic fibers. Supported by NIH grant 15114 to R.S.Z.

CALCIIUM-ACTIVATED POTASSIUM CHANNELS REGULATE SYNAPTIC TRANSMISSION AT THE FROG NEUROMUSCULAR JUNCTION.

R. Ricklofs and M. P. Chartres, MRC Group, Physiol. Dept., Univ. of Toronto, Toronto, Canada M5S 1A8.

Transmitter release is steeply related to Ca2+ concentration. The size of the Ca2+ transient is modulated by the duration of the presynaptic action potential (AP). We tested the role of Ca2+ -activated K+ channels (IKcals), in the regulation of transmitter release at the frog neuromuscular junction (NMJ). The motor nerve was stimulated and transmitter release was monitored using intracellular post synaptic recordings and specific IKcals channel blockers. Charybdotoxin (CTX, 2 nM), but not apamin (50 nM), increased release (163±6% ± 58.7%) without affecting MEPP frequency and amplitude. Similar results were obtained in low and normal Ca2+ concentrations. CTX (2 nM) had no effect on release after a membrane permeant Ca2+ buffer (DMBAPA-AM) was loaded in the nerve terminal. This indicates that the toxin acts primarily on Ca2+ -activated channels. Paradoxically, the intracellular Ca2+ buffer first induced a transient increase of transmitter release (26.8±3% ± 6.3%) before the decrease occurred. This increase did not occur when the loading was performed in presence of IKcals blockers (CTX 2 nM, Ba2+ 2 mM) indicating that opening of IKcals channels is reduced due to the attenuation of the Ca2+ transient by the buffer. The early increase of transmitter release did not occur when EGTA-AM, a Ca2+ buffer with similar affinity as BAPTA but slower forward rate constant, was used. We conclude that IKcals channels are an important regulator of synaptic transmission at the frog NMJ, where they cause spike narrowing, and are probably clustered close to the Ca2+ channels at release sites. Supported by MRC Canada.

COMPARISON OF THE ACTIONS OF BACLOFEN AND ADENOSINE AT PRESYNAPTIC RECEPTORS IN HIPPOCAMPAL SLICE CULTURE.

Scott M. Thompson, Helmut Haas, and Beat H. Gabieler. Brain Research Institute, Univ. of Zurich, 8029 Zurich Switzerland.

We have used intracellular recordings from CA1 to characterize the mechanism by which baclofen (BAC) and adenosine (ADO) block synaptic transmission in the hippocampus. BAC, but not ADO, blocked the amplitude of monosynaptic EPSPs (mIPSPs) elicited in the presence of CNQX and D-APV, suggesting that inhibitory terminals do not possess ADO receptors. Both substances reduced the amplitude of isolated EPSPs elicited with mossy fiber stimulation. BAC and ADO reduced mIPSPs but did not affect mEPSPs. This indicates that baclofen and adenosine do not act presynaptically by depressor post synaptic hyperpolarization in pertussis toxin-treated cultures, but both substances could still block EPSPs. However, BAC had no effect on mIPSPs. Stimulation of the stratum oriens did not reduce all presynaptic actions of both BAC and ADO. The post synaptic hyperpolarization elicited by BAC was also reduced, but the hyperpolarization elicited by ADO was not. Barium (1 mM) blocked the post synaptic hyperpolarizations elicited by both substances, as well as the action of BAC on mIPSPs. However, both ADO and BAC were able to depress EPSPs in the presence of barium. These results suggest that the presynaptic action of baclofen at inhibitory synapses is the same as the postsynaptic action: an increase in a G-protein mediated, barium-sensitive K+ conductance, which prevents GABA release by hyperpolarizing the terminals. The actions of baclofen and adenosine at excitatory axon endings must be mediated by some other mechanism.

532.21 INTRACELLULAR Ca2+ MEASUREMENTS WITH FURA-2 IN PRESYNAPTIC TERMINALS IN BULLFROG SYMPATHETIC GANGLIA.

V. Y. Peng and R. S. Zucker. Dept. of Molecular and Cell Biology, Univ. of California, Berkeley, CA 94720.

To investigate the Ca2+ dependence of neurotransmitter release in bullfrog sympathetic ganglia neurons, where the preganglionic C fibers release both ACh and LHRH, a method was developed to fill the presynaptic terminal boutons with Fura-2. Fura-2 appears to be transported to the terminal boutons via a fast method. Typically, at least 30 surface cells possessed filled boutons. For a given neuron, up to 50 individual boutons were observed. Fura-2 concentration in boutons was 100-500 μM. Ca2+ elevation in synaptic boutons due to a single stimulus to the presynaptic fiber can be detected by a photomultiplier. Most boutons had a resting Ca2+ concentration of ~30 nM. Only when the resting Ca2+ was ~70 nM, was there spontaneous release of ACh, which was measured as an increase in Ca2+ concentration of ~50 nM. The effect of ACh and LHRH on Ca2+ concentration was determined. For boutons on C neurons, the LHRH receptor antagonist (D-Phe1, D-Pro2, D-Trp4, 6)-LH-RH, 20 μM) decreased the Ca2+ elevation evoked by stimulation to the preganglionic fibers. Supported by NIH grant 15114 to R.S.Z.

532.22 RELATION OF PRETERMINAL NODES AND TERMINAL MORPHOLOGY TO DEPOLARIZATION OF THE MAMMALIAN MOTOR NERVE TERMINAL.

S. Shankar1 and N. Robbins2. Departments of 1 Biomedical Engineering and 2Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

The mammalian motor nerve terminal (NT) is thought to be passively depolarized by inward Na+ currents from preterminal nodes of Ranvier. The spacing of nodes, morphology of the NT arbor, and distribution of ion channels in the nerve membrane may affect the depolarization of the terminal, and thus its function. Aggregates, in particular, frequently exhibit NT constrictions that might decrease the current to distant branches. Since direct measurement of the NT voltage is not currently feasible, these issues were approached by morphologic and computer-modelling studies. The shapes of mouse motor NTs and their preterminal axons were determined with vital fluorescent stains. Discrete cable models of these terminals were constructed from the morphologic data and from published work on ion channel density, distribution, and kinetics. Membrane voltages and currents were computed during simulations over a range of electrotonic parameters. The clone (30-60 μm) spacing of the preterminal nodes resulted in a high safety factor for conduction, so that NT action potentials (AP's) occurred even after block of Na+ channels in two preterminal nodes. Decreasing the internodal spacing did not significantly increase the current flow to the terminal, and the measured internodal distances were not correlated with the size or input impedance of the NT. Conduction through the NT was modelled as purely passive. Even for small values of leak resistance (10 Ω cm2), small NT's were electrically compact. However, in large NT's, especially those with constrictions, AP amplitudes declined towards the distal ends. We conclude that preterminal internodal spacing provides safety of transmission, but is not fine tuned to match NT impedance. Also, in the passive case there are inhomogeneities in the depolarization of some NT's. Supported by NIA AG01015-07 and NIA AG08886.
533.1 SIMULTANEOUS PATCH-CLAMP RECORDING AND CONFOCAL IMAGING OF Ca2+ INFLUX INTO CA1 NEURONES OF THE RAT HIPPOCAMPUS.


Brief exposure to high concentrations of kainic acid produces a marked fluorescence increase in CA1 pyramidal cells in area CA1 of the rat hippocampus. Cells about 100 μm below the surface of the slice are recorded using whole-cell patch-clamp techniques, and high concentrations of kainic acid are applied through the patch pipette. Increased fluorescence is observed in both apical and basal dendrites, as well as in the soma. This increase is transient, with a rapid return to baseline fluorescence levels. The mechanism of this Ca2+ influx remains to be determined.

533.2 Confocal Imaging of Calcium Transients and Whole-Cell Recording from Pyramidal Cells in Area CA1 of Rat Hippocampal Slices.

R.J. Adams and T.J. Segalowski. Computational Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Calcium transients are considered to be important mediators of synaptic plasticity in the hippocampus and elsewhere in the nervous system. We are using confocal microscopy to observe changes in intracellular calcium concentration in pyramidal cells in area CA1 of 400μm thick slices of rat hippocampus. Cells about 100μm below the surface of the slice are recorded using whole-cell techniques using electrodes of about 5M2 resistance. The recording electrode filling solution includes fluo-3 as a fluorescent indicator of calcium ion concentration. Low noise electrical recording with simultaneous high temporal- and spatial-resolution imaging can be achieved with this technique.

Synaptic stimulation from axons of the Schaffer collateral/commissural pathway promotes calcium transients in the dendrites and soma of these cells. Changes in intracellular calcium in response to a single excitatory postsynaptic action potential, the firing of a single action potential, can be detected in the proximal dendrites and soma.

533.3 CONFOCAL IMAGING OF CHANGES IN SYNAPTIC STRUCTURE IN LIVING HIPPOCAMPAL SLICES.


We have investigated whether morphological changes occur in association with changes in physiological function. Synaptic structures are labelled by microinjection of a concentrated oil solution of Dil into the superfused slice; dye droplets are removed after 30 min. Using the technique of dye percursor rapid, and often intense, labelling of dendritic spines and axon terminals. Viability of labelled neurons is assessed by ethidium bromide exclusion. Field potentials from labelled regions are normal, and LTP can be induced by electrical or chemical stimulation. Brief exposure to high concentrations of kainic acid can induce large changes in dendritic shaft and spine morphology; smaller and inconsistent changes can be seen in control slices and after induction of LTP by elevated extracellular calcium.

533.4 ANALYSIS OF THE VARIABILITY OF EVOKED SYNAPTIC CURRENTS DURING LONG-TERM POTENTIATION (LTP) IN HIPPOCAMPAL SLICES.


High-frequency stimulation of the Schaffer axons, often inducing the A current transient, decreases afterhyperpolarization (aAHP) and mAHP associated with the A current and M current. Intracellular recordings from CA1 neurones (Vm = -77 ± 2 mV; n = 102 cells) showed that the decrease in the aAHP lasted about 30 s and was enhanced by application of 4-aminopyridine and 3,4-diaminopyridine, and by depolarization of the postsynaptic cell immediately prior to high-frequency stimulation. Conversely, the decrease in the mAHP was more variable and, on average, a decrease in CV was observed (15 cells). Since the CV was unchanged, our results favor the hypothesis that LTP induced by low frequency pairing is expressed postsynaptically. This interpretation however depends on several assumptions which may not all apply for synapses onto CA1 pyramidal cells (Kro et al., Nature 350, 282).

533.5 THE EFFECT OF LONG-TERM POTENTIATION ON MINIATURE SYNAPTIC CURRENTS IN HIPPOCAMPAL SLICES.


We have used whole-cell recording from guinea pig slices to analyze spontaneous excitatory postsynaptic currents (EPSCs) before and after LTP. Cells were voltage-clamped at -80 mV and EPSCs were evoked with electrical stimulation of afferent fibers at 0.2 Hz. LTP was induced by pairing 0.2 Hz stimulation with depolarization of the postsynaptic cell to 0 mV for 2.5 min. Stimulation produces a decrease in the size of EPSCs to nearly double with little change in shape. Following LTP induction, the decrease in the size of EPSCs is detected in 6 of 7 cells. In a second set of experiments we used a glass pipette, filled with 0.5 M sucrose in normal Ringer, to evoke miniature EPSCs (mEPSCs). This preparation was sensitive to the transmitter.

533.6 CHANGES IN NEURONAL EXCITABILITY DURING LONG-TERM POTENTIATION.


High-frequency stimulation of the Schaffer axons, often inducing long-term potentiation, decreased the afterhyperpolarization (aAHP) and mAHP associated with the A current and M current. Intracellular recordings from CA1 neurones (Vm = -77 ± 2 mV; n = 102 cells) showed that the decrease in the aAHP lasted about 30 s and was enhanced by application of 4-aminopyridine and 3,4-diaminopyridine, and by depolarization of the postsynaptic cell immediately prior to high-frequency stimulation. Conversely, the decrease in the mAHP was more variable and, on average, a decrease in CV was observed (15 cells). Since the CV was unchanged, our results favor the hypothesis that LTP induced by low frequency pairing is expressed postsynaptically. This interpretation however depends on several assumptions which may not all apply for synapses onto CA1 pyramidal cells (Kro et al., Nature 350, 282).
AVP-INDUCED POTENTIATION AND DEPRESSION OF EVOKED POTENTIALS IN THE DENTATE GYRUS IS REGULATED BY CALCIUM.


Vasopressin (AVP) has been shown to modulate memory processes (Brinton & McHenry, 1989). Rat hippocampal slices, maintained in a potassium-free solution and maintained with humidified oxygen mixture, were used to assess the effect of AVP on synaptic transmission. Field potentials were evoked by a bipolar electrode in the perforant path, and the responses were recorded by an extracellular microelectrode in the granule cell layer of the dentate gyrus. AVP (500 nM) applied in the perfusion solution has been found to have dual effects on responses of cells in the dentate depending on the external Ca. In 1.5 mM Ca, AVP potentiated the evoked response as measured by the change in population EPSP slope by 31.42±1% (t=5.72, p<0.001). In 2.5 mM Ca, AVP depressed the evoked response by 20.52% (t=5.37, p<0.007). Both the potentiation and the depression occurred within 5 min and lasted for more than 30 min after AVP application. Moreover, the changes in EPSP persisted following wash out of AVP. Thus, AVP-induced changes in hippocampal synaptic efficacy are long-lasting and are regulated by extracellular calcium.

Supported by the McNichol Foundation to RPT and NIH (MB64836) to RBR.

MODERATE CONCENTRATIONS OF POTASSIUM CHANNEL BLOCKERS INTERFERE WITH HIPPOCAMPAL LONG-TERM POTENTIATION.

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Several experiments suggest that maintenance of long-term potentiation (LTP) includes mechanisms located remote from the postsynaptic induction site. One possibility to convey signals over some distance is by means of ionic concentration changes. In order to test the hypothesis that shifts in potassium concentrations may be a crucial factor we investigated the effect of the potassium channel blockers tetraethylammonium (TEA) and barium (Ba²⁺) on the induction of LTP in the CA1 region of rat hippocampal slices. We observed that moderate concentrations of TEA (5-10mM) or Ba²⁺ (0.5-5mM) reliably and reversibly prevented the induction of long-term synaptic enhancement following tetanic stimulation. As bath application of these drugs acts on both, neurons and glial cells, we intracellulary recorded responses of both cell types to different stimulation. We observed increased polarization in pyramidal cells, which should indeed facilitate the induction of LTP. In contrast, the responses of identified astroglial cells were clearly diminished in the presence of TEA or Ba²⁺. These data accord with the hypothesis that activation of glial cells is an integral step in long-term plasticity. Supported by BMFT 316902A5.

Basal vs apical-dendritic LTP in hippocampal CA1 following commissural stimulation in the anesthetized rat.


In urethane-anesthetized rats, basal vs. apical-dendritic long-term potentiation (LTP) in CA1 was studied following stimulation of the contralateral CA3 and different layers of contralateral CA1 (str.oriens, pyramidale or radiatum/molecular). Average evoked potentials were recorded at 50 µm depth in intervals and analyzed by a one-dimensional current-source-density (CSD) before and after tetanus. Single test pulses evoked excitation(s) at both the basal- and apical-dendrites of CA1. Potentiation of LTP (IPG) was observed 15-30 minutes later by 10 pulses (20 Hz) of the contralateral CA3 str. oriented yields strong basal: (154.6±9.8, n=8) but little apical-dendritic sink (106.2±6.4, n=8). Both basal and apical-dendritic sink (103.2±19.6, n=6) and a depression of the apical-dendritic sink (83.3±7.5, n=6). Tetanization of str. pyramidale yielded a strong LTP of 100 μA (143.1±15.8, n=7) sinks. Tetanization of CA3 showed a strong basal-dendritic (130.0±14.4) and weak apical-dendritic sink increase (109.9±10.3, n=11). Test responses evoked by CA1 str.radial stimulation after the CA3 tetanization revealed heterodynamic depression of both basal: (75.9±10.5, n=7) and apical-dendritic (72.3±9.9, n=8) sinks. The CSDs indicated that both basal and apical dendrites are activated by hippocampal commissural stimulation, in some cases with more apical- than basal-dendritic excitation. However, the basal dendritic synapse seems to possess a greater propensity for LTP than the apical dendritic synapse, as in the behaving rat. Leung, Neurosci. Ansh. 16:652. Supported by Upjohn, NSERC.
533.13
TIME-COURSE AND PLASTICITY OF RAT HIPPOCAMPAL CA1 PYRAMIDAL CELL RESPONSES TO INPUT FROM RECURRENT COLLATERALS EXAMINED BY WHOLE-CELL RECORDINGS. R. L. Landon, J. Johnson, and G. B. Alkon. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pgh., PA.

Pyramidal cells in the CA3 field of hippocampus make direct excitatory synapses onto each other. We have studied these (1) as a mechanism whereby neuronal activity is synchronized, and (2) because studies of LTP of responses in CA3 depend on synchronization of recurrent collaterals and mossy fiber inputs. The latencies and rise-times of the former have not been well-studied and may provide criteria for such discriminations. To evoke EPSCs via collaterals in CA3B pyramidal cells we applied 1 Hz stimulation of CA3C, with, interposed upon the CA3b/CA3c border, a cut extending 125 to 200 μm radially from the alveus. In 5 out of 11 cells, the rising phase (10 to 95%) of the EPSC lasted between 1.4 to 3.0 msec. EPSCs with longer rising times often exhibited multiple inflections, suggesting temporal dispersion of inputs. The mean latency to EC input was 2.7 msec (S.D. = 0.4, range = 1.9 to 3.2) for CA3B cells which were between 750 and 970 μm from the stimulus site (n = 10). By comparison, direct conduction over this same distance by mossy fiber should require: 1.4 to 2.0 msec (at 33°C), based on the latencies of antidromic responses in n. granulosum, evoked by stimulation of the z. lucidum. To induce LTP, we applied 100 Hz trains at least 30 min after break-in, with cells clamped at -70 mV; presumably these conditions resulted only in LTP expressed polysynaptically and not on inputs to the cell recorded. Although late phases of the EPSC exhibited the greatest potentiation, nevertheless, the initial 3 msec of the EPSC was enhanced appreciably. The time-course of responses of CA3B pyramidal cells to inputs from collaterals can be rapid, and activity-induced potentiation of the collateral connections can affect early phases of the EPSC to collaterals. Supported by NINDS 524238 and NIMH M014156.

533.15
ASSOCIATIVE LONG-TERM POTENTIATION IN THE HIPPOCAMPUS USING ANTIDROMIC STIMULATION AS THE CONDITIONING TETANUS. I. M. Lester and T. 1. Neurosciences. Computational Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037.

Associative long-term potentiation (LTP) is a long-lasting increase in synaptic response produced when a stimulus insufficient to cause LTP is paired with a conditioning tetanus. In area CA1 of the hippocampus, associative LTP is mediated by depolarization of the postsynaptic membrane which relieves the Mg2+ block of the N-methyl-D-aspartate (NMDA) type of glutamate receptor. A post-antidromically-evoked action potential in the soma of CA1 pyramidal neurons depolarizes the postsynaptic membrane in dentrites enough to prime the NMDA receptor.

Extracellular recordings were obtained from the stratum lucidum of rats with electrodes in the CA1 and CA3 fields. Recording electrodes were placed in CA1 and used to explore for potentials in CA1 due to a single cutaneous input to CO1. The ischemic degree of the CA1 field is expressed via the stratum lucidum to the CA1 field (S.L. to CA1). The extracellular recordings were obtained with two electrodes: one placed in the CA1 stratum radiatum and the other used to explore, with 50 μm steps, the stratum lucidum. The data were stored in the computer and the one-dimensional CSD was calculated. The LTP was induced with short burst (4 pulses at 100 Hz), repeated 10 times with 200 ms of interval. In some experiments a 6 sec of interburst interval was used. The input-output curves were also computed before and after LTP. The results showed that in some experiments an early sink (ES) in pAD after induction of LTP appeared. Stimulation at 6 sec of interburst interval showed LTP and ES in pAD while no modification was observed in dAD. The modification in pAD was not induced by the APV (50 μM) perfusion following LTP induction. The data suggest that the antidromic stimulation inducing LTP modifies pAD properties of pAD increasing depolarizing currents and support the hypothesis of a second (dendritic) component in the expression of LTP. This modification is not maintained by NMDA receptor.

533.17
LONG-TERM POTENTIATION CANNOT BE INDUCED IN THE CA1-FIELD OF HIPPOCAMPUS OF MICROCEPHALIC RATS. Kambara, K.1, Kamitani, T.1, Urban, I.J.A., de Graan, P.N.E.,1 Carabelli, F.2, Di Luca, M.3, and Gessa, G.1, 4, 5, 6, 7.

1 Rudolf Magnus Institute, University of Utrecht, The Netherlands. 2 Institute of Neurology, University of Milan, Italy. 3 Department of Psychology, Yale University, New Haven, CT 06520. 4 Department of Neurochemistry, Psychopharmacology, Facultas Medicinale, University of Padua, Italy. 5 Institute of Pharmacology, University of Florence, Italy. 6 Department of Psychiatry, University of Texas, U.S.A. 7 Department of Pharmacology, University of Milan, Italy.

Injection of methylazoxy methanol (MAM) to pregnant rats produces in offspring a.o. microencephaly, hypoplasia in the cerebral cortex and CA1 field of the hippocampus, it is difficult to excite the mossy fiber synapse selectively when using extracellular microstimulation. For example, if the stimulating electrode is placed either in the stratum granulosum or in the stratum lucidum, it is possible to activate not only mossy fibers, but also also the perforant path, commissural fibers, CA3 recurrent collaterals, and local inhibitory circuits. We have used previous characteristics of mossy fiber synapses (Brown & Johnson, 1983; Williams & Johnston, 1989) to devise a set of minimal criteria for identifying isolated mossy fiber excitatory postsynaptic currents (EPSCs). These include: fast rise time (under 3 ms), voltage independence of the decay time constant, small dephasing at high-frequency stimulation. These criteria are not easy to satisfy. Slower currents of unknown origin as well as polysynaptic contamination are much more frequently observed. Using whole-cell recording methods, which has improved our signal-to-noise ratio by a decade, it is possible to dissect more components of evoked events. We have noted that slow small currents, which would be below the noise level using intracellular methods, are often observed in response to mossy fiber stimuli. Criteria for isolating mossy fiber events should be employed regularly, since failure to obtain pure mossy fiber events may lead to significant interpretation errors. (Supported by NIMH and NIH).

533.18
HETEROSYNAPTIC LONG-TERM POTENTIATION IN THE LATERAL ENTORHINAL CORTEX: AND EXTRA AND INTRACELULAR STUDY ON THE ISOLATED IN VITRO BRAIN PREPARATION. Marigo, A. Giorgi, and Rodoletto, B. Laboratorio di Neurofisiologia, Istituto di Neurologia, University of Milan, Italy.

A study of the properties of LTP in the entorhinal cortex (EC) (Alonso, de Curtis and Linas; PNAS, 87, 9280-94, 1990) demonstrated that a non-Hippocampal potentiation can be evoked in the layer II neurons by direct stimulating the pyramidal fibers, suggesting that postsynaptic mechanisms are operant in this form of LTP. The ability to induce heterosynaptic LTP was also demonstrated by following a high frequency stimulation of a separate input converging on the same cell population has been described (Bardier and Barnieuene, Synapse, 4, 132, 1989). Utilizing the isolated brain preparation maintained in vitro by arterial perfusion (Linhas, Muhlethaler and Walter; J. Physiol., 414, 16, 1989), we tested both extra and intracellularly the possibility of heterosynaptic enhancement of the entorhinal population of layer II in the EC. Two separate inputs converging on the apical dendrites of these cells were studied. The EPSPs evoked by both stimulation produced an enhancement of the EPSP amplitude. Input was induced either by a theta-frequency tetanic stimulation of the one input or by intracellular postsynaptic manipulations. In both cases heterosynaptic and non-Hippocampal potentiation of the responses evoked by both stimuli were obtained. In contrast, short-term post-tetanic potentiation (or depression) was observed only in the response to the tetanized input. Supported by NIH grant NS12742.

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THURSDAY PM

1330 LONG-TERM POTENTIATION: PHYSIOLOGY AND PHARMACOLOGY III

1330.14
ISOLATED MOSSY-FIBER SYNCYNTIAL CURRENTS IN CA3 PYRAMIDAL NEURONS. Z. Xiang, A.C. Nobr, and T.H. Brown. Dept. of Psychology, Yale University, New Haven, CT 06520.

The mossy-fiber synapse is particularly inviting to optical and experimental examination of plasticity due to its large size and extracellular proximity to the soma. Because there are many synaptic systems present in the CA3 region of the hippocampus, it is difficult to excite the mossy-fiber synapse selectively when using extracellular microstimulation. For example, if the stimulating electrode is placed either in the stratum granulosum or in the stratum lucidum, it is possible to activate not only mossy fibers, but also also the perforant path, commissural fibers, CA3 recurrent collaterals, and local inhibitory circuits. We have used previous characteristics of mossy fiber synapses (Brown & Johnson, 1983; Williams & Johnston, 1989) to devise a set of minimal criteria for identifying isolated mossy fiber excitatory postsynaptic currents (EPSCs). These include: fast rise time (under 3 ms), voltage independence of the decay time constant, small dephasing at high-frequency stimulation. These criteria are not easy to satisfy. Slower currents of unknown origin as well as polysynaptic contamination are much more frequently observed. Using whole-cell recording methods, which has improved our signal-to-noise ratio by a decade, it is possible to dissect more components of evoked events. We have noted that slow small currents, which would be below the noise level using intracellular methods, are often observed in response to mossy fiber stimuli. Criteria for isolating mossy fiber events should be employed regularly, since failure to obtain pure mossy fiber events may lead to significant interpretation errors. (Supported by NIMH and NIH).
LONG-TERM POTENTIATION AND DEPRESSION OF THE EXCITABILITY OF THE CORTICOSTRIATAL TERMINAL FIELD. M. Garcia-Nunez, E.J. Young and P.M. Groves Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, England We have found that long-lasting changes in the excitability of glutamatergic corticostriatal terminals can be induced by tetanic stimulation. Antidromic responses, recorded from medial prefrontal cortex were elicited by stimulation of the cortical terminal field in urethane anesthetized rats. Excitability was assessed by determining the occurrence and direction of the long-lasting excitability change. This work was partially supported by grants from NIDA and the Office of Naval Research.

THURSDAY PM LONG-TERM POTENTIATION: PHYSIOLOGY AND PHARMACOLOGY III

533.19
IDENTIFICATION OF A REGION WITHIN GLUTAMATE RECEPTOR SUBUNITS THAT CONTROLS CALCIUM PERMEABILITY. R. Dapprich, R.L. Hume and S.P. Homanics. Molecular Neurobiology Lab., The Salk Institute, La Jolla, CA 92037. The glutamate receptor clones, GluR1 and GluR3, when transcribed and expressed individually or together in Xenopus oocytes, assemble into receptors that exhibit marked rectification and Calculate permeability in response to kainate. Co-assemble of either subunit with GluR2, however, results in a receptor with a linear i-v curve and greatly reduced calcium permeation (Hollmann et al., Science 1991). We have constructed subunit chimeras and point mutations to locate sites controlling the permeation characteristics of these glutamate receptors. A key residue was identified, R607 in GluR2 and its equivalent, Q612, in GluR3 (mutated from the GluR1 template). Intercrossing of amino acids at this position in GluR2 and GluR3 switched their phenotypes. Thus, GluR2(R607Q) expressed alone was active in contrast to the parent GluR2, which showed little activity, and GluR3(R607Q), behaved functionally like GluR3 in that its i-v curve displayed marked inward rectification in normal (high Na) solutions. In solutions containing low Na and high Ca or Ba, this construct responded to kainate with an inwardly rectifying current. Conversely, GluR3(Q612R) had a GluR2 phenotype since it was inactive when expressed alone but when co-expressed with GluR1 or GluR3 assembled into a receptor with linear i-v curve in normal solutions and outward Goldman rectification in low Na, high Ba solutions. Other mutants of GluR3 around position 612 produced similar but not identical effects. These findings suggest that the region identified is in or near the ion permeation path and is an important determinant of ion permeability through non-NMDA receptor channels.

533.20 LONG-LASTING MODIFICATION IN SYNAPTIC EFFICACY AT PRIMARY AFFERENT SYNAPSES WITH NEURONS IN RAT SUPERFICIAL SPINAL DORSAL HORN. R. Canolty, T. Rinne, J.A. Jeneson, S. Mino, C. Poulopoulou, and L.M. Nowak. Physiology and Pharmacology, Iowa State University, Ames, IA 50011. Although a great deal is known about long term potentiation (LTP) in the brain, the existence of a similar phenomenon in peripheral afferent synapses has not been demonstrated, as yet. We have investigated the excitatory synapse between small primary afferent fibers (Aβ and C) and neurons in the superficial laminae of spinal cord (DH) by intracellular recording from dorsal horn neurons in a transverse slice preparation of rat (25-39-days-old) spinal cord. Single electrical stimuli applied to the dorsal root evoked monosynaptic and/or polysynaptic excitatory postsynaptic potentials (EPSPs) in all DH neurons. Both types of EPSPs were blocked (n=13) by a new non-NMDA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (NBQX, 0.5-1.0μM for 2-4 min) indicating that a non-NMDA subtype of glutamate receptor mediates most of the fast EPSPS. However, the participation of a slower NMDA receptor-mediated component has also been shown. The depolarizations evoked by L-glutamate and L-aspartate were depressed by only 15-60% in the presence of NBQX (1-10μM). Brief, high frequency electrical stimulation of primary afferent fibers (100 Hz, 1 s) produced a long-lasting potentiation of primary afferent-evoked EPSPs that appeared to be monosynaptic. In addition, in several cells a long-term depression of monosynaptic excitatory transmission was elicited. The results demonstrate that distinct and long-lasting changes in synaptic efficacy can be induced by high-frequency stimulation of primary afferents and that these may be physiologically relevant for transmission and integration of sensory information, including pain. (Supported by NS 26502 and BNS 841 8042.)

ION CHANNELS: LIGAND-​GATED

534.1
GLUTAMATE RECEPTOR CHANNEL KINETICS IN EMBRYONIC DROSOPHILA MYOTONES. H. Chang* and Y. Kidokoro. Jerry Lewis Center, UCLA School of Medicine, L.A., CA 90024. Glutamate receptor channel kinetics were studied in embryonic Drosophila myotones formed in culture. These sugar myotones may be studied together with prolonged events. Long openings were interrupted by brief closures, thus showing bursting behavior. The burst histogram was fitted with three exponentials, the burst, intermediate and long component. The mean duration of long bursts increased with the glutamate concentration. The closed time histograms and between long bursts were fitted with two exponentials at low concentrations and three at high concentrations. The concentration dependent change in the burst duration, the mean duration and the relative amplitude of the long closed time component were fitted with a set of rate constants in the four state cooperative model in which two glutamate molecules bind to the receptor and open the channel. Generally, the scheme well predicted these changes but there were systematic deviations from the basic scheme.

534.2
THE DIVERSITY OF DIFFERING SUBSETS OF HIPPOCAMPAL NEURONS. C. Poulopoulou and L.M. Nowak. Department of Pharmacology, Cornell University, Ithaca, NY 14853. Excitatory amino acid (EAA) receptors are broadly classified as NMDA and non-NMDA types. Functional separation of the numerous non-NMDA receptors couples to ion channels has proved difficult because of poor pharmacological tools. Recent molecular biology studies indicate a large diversity non-NMDA EAA receptor channels which is likely to underly the diversity seen in electrophysiology. Thus, we are continuing our efforts to characterize non-NMDA receptor channels by measuring biophysical properties of kainate (5-200μM) activated channels in outside-out patches excised from forebrain neurons grown in dissociated cell culture for 10-35 days. Recording solutions contained (in mM): 150 NaCl, 2.5 KCl, 1.0 CaCl2, 1.0 MgCl2, 10 Hepes, pH 7.2; and 300 mM sucrose. Paradigm homogeneity was determined (in mM): 145 CsCl, 4 ATP-Mg, 10 EGTA, 10 Hepes-K(pH 7.2). Three different channels populations were identified. Most patches contained more than one channel type, but some showing mainly one class were obtained for all 3 populations. The brief (t < 0.8 ms) 30-40 pS channel appeared to desensitize rapidly. The 12-14 pS channel openings were interrupted by brief closures (T = 20-30 ms; T2 = 0.5 - 0.8 ms from noise). The 1 pS conductance (estimated from noise variance) had a main t of 20-40 ms (90% to 98% of the power density). 1 pS and 12-14 pS channels were activated by kainic acid, and Br-willardin. The 12-14 pS channel was more sensitive to inhibition by CNQX than the 1pS channel. Supported by NS 24467 to L.MN.

Methods based on the theory of stochastic point processes were applied to the study of the temporal pattern of activation of endplate channels. Single channel currents activated by acetylcholine (ACh, 4-20 nM) were recorded from muscarinic cells by means of whole-cell patch clamp recordings. The results obtained from the initial exposure of the patch to ACh, allowing us to observe the relaxation to steady-state of slow desensitization. The digitized records were searched for opening transitions, which were taken for point events and openings intervals (OIIs) were statistically analyzed in order to characterize the structure of the underlying point process. This analytical approach depicted two main subtypes of the receptor-channel desensitization process: (i) a decreasing trend in the frequency of openings, the rate of which increased with ACh concentration, and (ii) a tendency for openings to occur in clusters of bursts, also directly concentration-dependent. When several channels contributed to the current, the trend could be fit by maximum likelihood, assuming a non-homogeneous Poisson model. In addition, expectation density function estimates showed evidences of long term (* = 1 min) dependence between openings suggestive of a coupled regulatory process.


Ligand-gated single channel activity was recorded from rat brain membranes reconstituted into phospholipid bilayers formed on the tips of patch electrodes. Using cesium as the charge carrying ion, the reconstituted receptor exhibited properties of the NMDA receptor complex.

Addition of NMDA (20 μM to 100 μM) to the pipette solution produced channel openings. With glycine (20 μM to 100 μM) and strychnine (1 μM) present along with NMDA, channel conductance was 55 pS. The gating of this channel was independent of voltage in the absence of Mg²⁺. Addition of 100 μM Mg²⁺ resulted in increased channel bursting and voltage dependency of opening. Addition of the NMDA antagonist MK801 (100 μM) completely blocked channel opening. This method provides an opportunity to study the effects of the NMDA receptor-channel complex under tightly controlled experimental conditions. (Supported by PHS RR 007085-23.)
534.11 MAPPING OF THE LIGAND BINDING SITE OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS USING CHIMERIC SUBUNITS.

C. W. Lewejoh & S. Piattoni and J. W. Patrick. Division of Neuroscience, Baylor College of Medicine, Houston TX 77030.

A family of genes has been identified which encodes subunits of neuronal nicotinic acetylcholine receptors (nAChR). The α2, α3 and α4 subunits can each form functional nAChR when co-expressed with β2 or β4 subunits. Each α-subunit contains a unique pharmacological properties. α3β2 is insensitive to blockade by neuronal bungarotoxin (NBT) (IC50>10μM), and is more sensitive to nicotine than to acetylcholine. In contrast, α3β2 is highly sensitive to NBT blockade (IC50<10μM), and is less sensitive to nicotine than to acetylcholine. Amino acid residues involved in NBT and nicotine sensitivity were mapped by generating a series of chimeric subunits composed of portions of α2 and α3, and co-expressing them with β2 for analysis. NBT sensitivity was localized to a region of α3 between I584 and I645. Two chimeric subunits constructed at glul95 of α3 (α2-glul95-α3 and α3-glul95-α2) formed receptors insensitive to NBT, suggesting that amino acid residues involved in NBT sensitivity flank glul95. Chimeric subunit α2-ile215-α3 formed a receptor more sensitive to nicotine than to acetylcholine while chimeric subunit α3-ile215-α2 formed a receptor less sensitive to nicotine than to acetylcholine. These results demonstrate that amino acid residues involved in nicotine sensitivity are located between the amino-terminus and ile215, and may flank glul95.

534.12 THE EXTRACELLULAR DOMAIN OF THE NEURONAL NICOTINIC SUBUNIT β4 DETERMINES THE PHARMACOLOGY OF RECEPTORS FORMED WITH α3.

Roger L. Popka, Robert Duvoisin* & Stephen F. Heinemann. Molecular Neurobiology Laboratory, Salk Institute, La Jolla CA 92037

The neuronal nicotinic acetylcholine receptor alpha subunit, α3, forms functional receptors when co-expressed with β4 subunits in Xenopus oocytes. The β2 and β4 subunits differ in pharmacological properties. α2β2 is less sensitive to blockade by neuronal bungarotoxin (NBT) (IC50>10μM), while α3β2 is insensitive to blockade by neuronal bungarotoxin (NBT) (IC50>10μM), and is more sensitive to nicotine than to acetylcholine. While the alpha subunits of nicotinic acetylcholine receptors are believed to contain the principal agonist binding sites for activation, in the case of neuronal nicotinic receptors, the beta subunit is also involved in pharmacology, as well as the kinetics of agonist binding and channel activation. For example, it has been shown that α3β4 receptors are resistant to neuronal bungarotoxin (α3-BTX), while α2β4 receptors are blocked by α3-BTX (Pellegrini, et al., 1989). Also, α3β4 receptors are effectively activated by cytisine, while α3β2 receptors have been reported to be 100 fold less sensitive to cytisine than to ACh (Lange & Patrick, 1991). In order to determine that portion of the beta subunits which may influence agonist and antagonist effects, we have created chimeric beta subunits which contain reciprocal exchanges between the β2 and β4 subunits in the first transmembrane domain. The chimeric subunits were constructed using PCR to create half subunit pieces that shared a common terminal sequence. A final PCR then used the region of overlap to prime the synthesis of full length chimeric subunits. NBT sensitivity was found to be modulated by the exchange of adjacent amino acids, and the remainder of the sequence was co-expressed with α3, receptors are formed which are resistant to neuronal bungarotoxin and are activated equally well by cytisine and ACh. The macroscopic concentration-response relationship of receptors formed with β4/2 also show the higher Hill slope that is typical for β4 containing receptors. Conversely, α3β2/4 receptors are blocked by β-tx, are relatively insensitive to cytisine, and have a lower Hill slope.

534.13 DIFFERENTIAL MODULATIONS OF GABA RECEPTOR CHANNEL BY POLYVALENT CATIONS. H. YAN and T. NARAHASHI. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611

GABA receptor channel complex is a multimeric receptor protein with an integral chloride channel. We report here that polyvalent cations modulate the GABA-induced chloride current in a differential manner. The currents were recorded from the rat dorsal root ganglion neurons in primary culture by the whole cell patch clamp technique. The mechanisms underlying the modulations were also studied. Among all the cations tested at a concentration of 1 mM, Cu2+, Zn2+, Co2+, Cd2+ and Pb2+ suppressed the GABA-induced current to different degrees whereas Mg2+ and Al3+ had no effect. Zn2+ and Cu2+ were most potent and abolished the GABA response almost completely. However, Mg2+ inhibited the response 2-3 fold. The modulations by polyvalent cations were reversible after washing with normal solutions. Suppression by Cu2+ and Zn2+ and augmentation by Mg2+ was voltage dependent. The GABA-induced holding potential of -60 mV. La3+, Cu2+ and Zn2+ all exerted their effects in a voltage-independent manner as they reduced the responses to glycine. Competition studies showed that Cu2+ and Zn2+ did not compete with GABA for a binding site. These data suggest the existence of a cationic regulatory site distinct from the GABA site at or near the external orifice of the chloride channel.

534.14 INHIBITORY NEUROTUMMER RECEPTORS PRESENT IN CULTIVATED RAT MEDULLARY NEURONS.

C. A. Lewis and D. Faber. Neurobiology, SUNY at Buffalo, Buffalo, NY 14214.

Whole-cell current responses to bath application of glycine, β-alanine, taurine, and GABA were studied in medullary neurons cultured from embryonic rats. Two-component responses were evoked by bath application of agonist, one component which desensitized and another which did not. The two current components have different dose-response characteristics with the nondesensitizing component being activated more effectively at lower concentrations than the desensitizing one and also reaching its peak at lower concentrations. For all agonists tested, both current components have different strychnine sensitivities, with the desensitizing component being more sensitive. The complete occlusion between the responses to glycine and taurine and glycine and taurine suggest that these agonists activate the same receptors. Taurine and β-alanine are less potent agonists than glycine with relative potencies of 0.01, 0.01 for glycine: β-alanine: taurine. On the other hand, there was incomplete occlusion between the responses to glycine and GABA, suggesting that these agonists activate both similar and different populations of receptors. In confirmation, the receptor population activated by either glycine or GABA was blocked by both strychnine and picrotoxin. In conclusion, there appear to be at least 3 populations of inhibitory neurotransmitter receptors—GlyR, GABAR, and Gly/GABAR.

534.15 SINGLE-CHANNEL PROPERTIES OF NEWBORN RAT SPINAL CORD GLYCINE RECEPTORS EXPRESSED IN XENOPUS OCYTES.

Quoc Thang Nguyen, Andres Morales and Ricardo Miledi. Laboratory of Cellular and Molecular Neurobiology, Dept. of Psychobiology, University of California, Irvine CA92715 USA

Using the noise-analysis technique, we measured the single-channel characteristics of glycine receptors (expressed in Xenopus oocytes) by heavy (H) and light (L) mRNA isolated from the newborn rat spinal cord (cf. ref. 1 & 2). Oocytes were injected with H or L mRNA and recorded four to six days after injection. Cells were clamped at -60mV and perfused with 1-2x10-5 M glycine. The mean open time was 0.5±0.18 ms, near fold increase over the value found in the adult (ref. 2). In addition, some spectra could be fitted by one Lorentzian; some by two; the mean open time was 50.9+/-6.3ms, a near threefold increase over the value found in the adult. The macroscopic concentration-response relationship of receptors formed with β4/2 also show the higher Hill slope that is typical for β4 containing receptors. Conversely, α3β2/4 receptors are blocked by β-tx, are relatively insensitive to cytisine, and have a lower Hill slope.

534.16 MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF A SEROTONIN-GATED ION CHANNEL.


Serotonin is a ubiquitous neurotransmitter whose diverse functions are mediated by activation of pharmacologically distinct receptor subtypes. Although most serotonin receptors appear to transduce extracellular signals by interacting with G-proteins, the SHT3 subtype of serotonin receptor (SHT3R) was recently shown to incorporate a ligand-gated cation-selective ion conductance. We have isolated a functional clone encoding a SHT3R receptor (SHT3R-A) by screening a neuroblastoma cDNA expression library for serotonin-gated currents. The isolated clone encodes a single protein that has many of the predicted secondary structural features shared by other ligand-gated ion channels (e.g. the nicotinic acetylcholine receptor). When the cloned receptor is expressed in oocytes, the pharmacological and electrophysiological properties of the serotonin-gated current are largely consistent with the properties of the SER3. Potential and selective SHT3R antagonists such as ICS 205-930 block the serotonin-gated current at nanomolar concentrations. In agreement with previous reports, the current passes through a non-specific cation conductance that is modulated by the external concentration of divalent cations. However, we also find that 1-V plots of the serotonin-gated current display a region of negative slope conductance that is sensitive to the external divalent cation concentration and that resembles the voltage and divalent cation mediated conductance changes observed in the NMDA subtype of excitatory glutamate receptor.

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The medial pontine reticular formation (mPRF) plays an important role in REM sleep, in which the starlike reflex in which Na+ channels of the locus coeruleus display firing activity inversely correlated to the expression of REM sleep. Noradrenergic neurotransmission is important in behavioral state control and influences the appearance of REM sleep. The action of norepinephrine (NE) on neurons of the mPRF was examined in rat brain slices in vitro using intracellular electrophysiological methods.

Application of NE to mPRF neurons results in a depolarization of 74% of the neurons tested (n=28). NE is mimicked by the α, agonist phenylephrine (PE, n=17). NE and PE inward currents were accompanied by a decrease in membrane conductance. The reversal potential of the inward current elicited by PE corresponded to that of K+ and was sensitive to blockers of K+ in a Nernstian manner. The current-voltage relation recorded during voltage ramp commands indicates a strong voltage dependence of the PE sensitive current. The conductance change elicited by PE is greater at depolarized membrane potentials. The mechanism of the PE induced conductance change is under study.

These results support the conclusion that NE acting at α receptors causes a decrease in potassium conductance. The potassium conductance(s) involved in the action of NE has not yet been identified.

DOMAIN STRUCTURE OF THE INOSITOL-1,4,5-TRISPHOSPHATE RECEPTOR. G.A. Mignerv*, C. L. Newton* and T.C. Südhof Howard Hughes Medical Institute, Department of Molecular Genetics, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75233

The inositol-1,4,5-trisphosphate (InsP3) receptor is composed of a homotetramer of highly conserved 313Kd subunits which contain eight membrane spanning regions in the COO-terminal fourth of the protein. The structural features of the receptor were examined by autogonosis and transient expression in COS cells. Deletion of the eight membrane spanning regions results in the expression of a soluble, monomeric protein which binds InsP3 with an affinity and specificity similar to the wild-type receptor. This data indicates that the membrane spanning regions are essential for subunit oligomerization but do not contribute to the ligand binding site and that each receptor subunit contains an independent ligand binding domain. To identify the sequences comprising the binding site, OOO-terminal deletion mutants were constructed. InsP3 binding analysis on the truncated receptor proteins showed that the ligand binding region resides in the OOO-terminal fourth of the receptor subunit which is separated from the putative Ca2+ channel by over 100 amino acids. Gel filtration experiments demonstrate a large conformational change of the receptor as a function of ligand binding suggesting a mechanism by which ligand binding might cause channel opening. Taken together, these results allow each receptor subunit to be divided into an OOO-terminal ligand binding domain, a central coupling/regulatory domain and a COO-terminal cluster of membrane spanning regions which interact to form the receptor homotetramer and the Ca2+ channel.


Connexin (Cx) is an abundant gap junction protein in the developing nervous system; in the adult brain it is confined to specific cell types (e.g., pinealocytes and leptomeninginal cells) and is usually coexpressed with other connexins. To define the properties of Cx26 in stably transfected pC12 cells (a well-characterized, communication-deficient neuronal system) with Cx26 cDNA, using a plasmid containing a dexamethasone (dex)-inducible promoter, we stably transfected pC12 cells (presumably elongated transcripts including portions of the vector) were injected with G418-resistant colonies were injected with lucifer yellow and four dye-coupled colonies were separated for further analysis. Whereas mRNA encoding connexins 32, 43 and 26 were not detected in the parental cell line using Northern blot analysis, two bands (presumably elongated transcripts including portions of the vector) were found to hybridize to the Cx26 probe in the transfected colonies. Immunofluorescence using connexin-specific antibodies failed to reveal connexins 26, 32 or 43 in the parental line, but in the single transfected clone thus far analyzed, punctate Cx26 immunoreactivity was prominent. Junctional conductance was barely detectable in the parental line (0.03 ± 0.05 pS; n=19) but was moderate in dex-treated transfecants (4.0 ± 0.8 nS; n=41). Two populations of unitary junctional events were detected in Cx26 transfecants after dex treatment, suggesting that the latter event was seen in an untransfected dex-treated pC12 cell pair and in a pair of untreated pC12 transfecants, we attribute the smaller event to channels formed by the introduced Cx26. Cx26 is a well-coupled cell line in which neuronal phenotype can be induced should allow biophysical analysis of Cx26 and illuminate the role of gap junctions in neuronal differentiation and function.

REGULATION OF Ca2+ RESPONSES TO ENDOTHELIN-1 BY VOLTAGE- AND RECEPTOR-GATED Ca2+ CHANNEL ANTAGONISTS AND DRUGS ACTING ON PHOSPHOLIPASE C AND PROTEIN KINST. J. Chan and D.A. Greenberg. Department of Neurology, University of California, San Francisco, CA 94110.

Endothelin-1 (ET-1, 5 nM) produced biphasic (peak and sustained plateau) elevations of free intracellular Ca2+ (Ca2+<sub>i</sub>) detected by fura-2 fluorescence in NG108-15 neuroblastoma x glioma cells. Both peak and plateau responses were attenuated by depletion of extracellular Ca2+. Peak responses were also reduced by the putative receptor-gated Ca2+ channel antagonist SKF9,6365 (30 μM), while plateau responses were abolished by 100 μM nmidemine. The phospholipase C (PLC) inhibitor U-73122 (1 μM) and the protein kinase C (PKC) activator phorbol 2-myristate 13-acetate (PMA, 1 μM) reduced peak responses by 80% and 35%, respectively, compared to responses in the presence of the inactive analogs U-73343 and 4a-PMA, in Ca2+-depleted buffer, both U-73122 and PMA abolished peak Ca2+ responses, while the PKC inhibitor staurosporine (100 nM) increased peak responses by 250%. These findings suggest that peak Ca2+ responses to ET-1 result from influx of Ca2+ through receptor-gated channels and PLC-activated mobilization of intracellular Ca2+, and are inhibited by PKC, while plateau responses involve influx of extracellular Ca2+ through dihydropyridine-sensitive, voltage-gated channels.

IONS CHANNELS: CELL FUNCTION


Work on insect muscle has shown that Diptera have a ouabain-sensitive Na,K-ATPase, whilst Lepidoptera have a ouabain-insensitive K<sup>+</sup>-pump. Using K<sup>+</sup>-selective microelectrodes, we have quantified the effects of various metabolic inhibitors on membrane potential (EM) and intracellular K<sup>+</sup> activity (aKi) in skeletal muscle of Phormia terranovae (Diptera) and Spodoptera exigua (Lepidoptera). The following inhibitors were used: dinitrophenol, cyanide, azide, rotenone, dicyclohexylcarbodiimide, salicylhydroxamic acid, ouabain, ethacrynic acid, vanadate, amiloride. Each inhibitor (0.1 mM) was applied for 60 mins, at 20°C and EM and aKi recorded continuously from different cells (20-50 cells; 5-7 insects).

Most inhibitors caused significant reduction of aKi (p<0.001) apart from ouabain and amiloride, which were ineffective in Spodoptera and Phormia, respectively. Strong inhibition by vanadate was common to both insects, suggesting that both have P-type ATPases. However, differences in ouabain and amiloride sensitivities suggest the molecular structures of the two pumps are different. All inhibitors except ouabain, caused significant depolarization of EM (p<0.001). For a given species, the inhibitor profiles of EM and aKi were largely similar, implying that changes in EM and aKi are closely linked.
Pterygodiscus brevis


535.3

The Perineural waveforms affected by dinoflagellate
Ptychodiscus brevis induced numerous fitz and cardio toxicity. The toxins in the dinoflagellate initially facilitated the transmitter
relaxing process followed by complete block of the neurotransmitter.

535.4

The toxins, brevetoxin-B, affected the population of cardiac calcium currents of the nerve terminal was studied by the perineural
waveform recordings of adult I.C.R. mouse (Mus musculus from Institute of Pharmacology, College of Medicine, National Taiwan
University, Taipei, Taiwan, R.O.C. The toxins in the dinoflagellate.

535.5

The perineural waveforms on the nerve terminal. The effect
of the toxin on the ion currents of the nerve terminal is as follows: slow calcium currents of the nerve terminal. The effect
of brevetoxin-B increased the calcium-activated potassium current and slow calcium currents of the nerve terminal. At this
concentration, the toxin had no effect on the fast calcium current and slow potassium currents. At higher concentration (1.1 µM), the toxin decreased all of the perineural waveforms on the nerve terminal. The effect may contribute to the human toxicity of the dinoflagellate.

535.6

Membrane properties and GABA responses of the

The trigeminal ganglia cells, isolated and cultured for 1-4 days, were examined with the whole-cell recording technique. The action potential showed a hump in the falling phase, with a half-duration of 1.6 ms and a hump when the membrane potential had been shifted to more positive levels. Hence, the TTX-sensitive K current seemed to be the major depolarizing the spike. The spike duration was prolonged with CsCl-filled electrodes, which was reduced by Co2+, but not the movement of the Ca current. Two types of the Ca current, L and H, were confirmed. These currents seemed to govern the injury pattern: with max firing rate reaching only about 70 Hz. Perfusion with GABA of 0.1-1 mM induced inward, mostly Cl, current while glycine had no effect. In some cells, GABA also reduced the Ca current moderately. Thus, GABA might exert presynaptic inhibition mediated by hyperpolarization and also probably by modulation of an intracellular signal transduction, at the proximal endings of the trigeminal nerve.

535.7

Intracellular recording and staining of terminal nerve

We have previously shown in the dwarf gurnard that terminal nerve (TN) cells are a major component of the GnRH system and that the TR-8-GnRH system is structurally independent from the prooptic/hypophyseal-GnRH system which facilitates gonadotropin-releasing hormone release. Thus, we have hypothesized that GnRH may function as an important neuromodulator (Ono and Ichikawa, 1990). A method of GnRH preparation has been developed to facilitate studies on the tonic and biochemical mechanisms of action of TN-GnRH neuromodulatory system. In this study, I examined the spontaneous electrical activity of TN cells using both extra- and intracellular recording techniques and determined the morphology of individual cells by intracellular injection of Lucifer yellow or biocytin. TN cells showed endogenous activities of either slow regular beating (1-6 Hz) or bursting patterns according to the physiologic peculiarities of the region of the brain (e.g., sexual maturity) of the fish. Anatomical observation of intracellularly-labeled TN cells revealed several specific branches that project to regions where we had previously demonstrated dense GnRH-immunoreactive fibers. The electrophysiological and morphologic properties of TN-GnRH cells suggest an advantageous for their possible role as a neuromodulator; the intrinsic membrane properties of modulating neurons change according to the animal's physiological conditions, and these modulator neurons in turn modulate neural activities in a wide variety of brain regions simultaneously via multiple axon branches.

535.8

Thalamic firing patterns are highly sensitive to variation in low threshold calcium channel: computer simulations of voltage and current clamp experiments. W.W. Lytton and T.I. Sejnowski, Salk Institute, La Jolla, CA 92037.

The low-threshold calcium channel (T channel) is believed to be critical in producing the anode break behavior characteristic of thalamic relay cells. We used computer simulation models to explore the influence of different models of this channel on responses to simulated current clamp. We first reproduced characteristic current/voltage curves under simulated voltage-clamp using a model of the high threshold calcium channel (L channel) combined with a model of the T channel obtained from either of two sources (Coulter et al., Physiol. Rev. 1989; 69:607-64; Cruelle et al., J Physiol. 1989).

Thalamic cells show a complex variety of responses under current clamp. Characteristic firing patterns include: 1) a single spike on a plateau in response to depolarization; 2) a single spike on a plateau followed by increased depolarization from a depolarized potential; 3) a burst of bursts of spikes as an anode break response following hyperpolarization (Lakeman and Linas, J Physiol. 1984; 357:277-297). We were able to simulate these firing patterns using a model that included 8 channels found in this cell type. Firing behavior was highly dependent on the details of the T channel model used and varied widely across different simulated conditions. These differences may reflect actual variation due to the different voltage-clamp techniques or actual differences due to species and developmental stage. Relative minor channel variations could be exploited in control mechanisms that alter the firing pattern of the cell.

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535.9 ESTIMATION OF PASSIVE ELECTROSTATIC PARAMETERS USING THE INVERSE FOURIER TRANSFORM TECHNIQUE TO STUDY ELECTROPHYSIOLOGIC EFFECTS ON GRANULAR CELLS. M. Paul D. Durand, G.L. Yuen and R. Chisholm*a. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Accurate extrinsic parameters of a neuron is essential for understanding the neuronal integration and information processing. The shunt cable model (Durand et al., 1982), which allows for different somatic and dendritic time constants was used to test the model the data generated cells and was modified to include an electrode artifact. The input impedance of the model and its voltage response to a current stimulus were calculated in the frequency domain, converted to the time domain using the inverse Fourier transform, and the parameters were estimated by nonlinear, least-square fitting of the model output to the voltage response to a current stimulus. Optimization of the objective function was obtained using the gradient method (NL530) algorithm. In order to quantify the consequences of acute changes in neuronal parameters such as the input resistance, dendritic and somatic time constants, resistances, and capacitances were obtained before and after exposure to low doses (50 mM) of ethanol and compared.

Intracellular responses to short hyperpolarizing current stimuli (0.3 msec, 2.5-7.5 nA) were recorded in 12 cells from hippocampal slices prepared from adult rats. The membrane time constant (τm) was found to decrease in ethanol by 24 %, while the somatic time constant (τs) increased by 27 %. The electronic length also increased in ethanol from (mean ± SD) Ls = 1.026 ± .12 to Ls = 1.202 ± .17, (p<.05). Although the mechanism(s) for these changes is unclear at this time, they may influence the neuronal integration in ethanol.

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The extramural cortex (EC) layer II is the major source of afferents to the hippocampus via the perforant path and receives inputs from the entire cortical mantle. It is also innervated by multiple subcortical structures including notably the cholinergic neurons of the basal forebrain (Alonso and Klink, J. Comp Neurol. 1986). The EC is believed to be a critical player in the modulation of the pyramidal-like cells (PLs) (Alonso and Limas. Soc. Neurosci. 1990). On the SCs, carbachol induced a modest depolarization, decreased the fast afterhyperpolarization and suppressed cell firing accommodation. It also reduced a subthreshold Na-current, input resistance and time constant of the action potential at the break of hyperpolarizing current pulses suggesting a cholinergic modulation of a subthreshold Na-conductance. The Na-dependant subthreshold oscillatory activity characteristic of the SCs also showed a small reduction in frequency and rhythmicity with carbachol. On the PLs, among other effects, carbachol drastically affected the cells firing behavior by inducing a slow rhythmic bursting pattern which was associated with a subtotal of the post-transafterhyperpolarizations by long-lasting plateau depolarizations. The results demonstrate a potentially strong influence of basal forebrain cholinergic inputs to the modulation of EC layer neurons' intrinsic excitability by which the processing of incoming afferent information in the hippocampus will be strongly affected (Supported by Canadian MRC).


The stellate cells from entorhinal cortex layer II display Na-dependant (inward) and Ca-dependent (outward) oscillatory activity that probably underlies the generation of the theta rhythm in this cortical area (Alonso and Limas, Nature, 1990). By using intracellular recordings in "in vitro" rat brain slices, we have investigated the intrinsic membrane properties that may contribute to generation of the oscillatory behavior. Following blockade of Na currents with TTX, analysis of the current-voltage relationships revealed a robust time-dependent inward rectification as well as a fast (non-transient) outward rectification which developed strongly at the oscillatory voltage level. Time-dependent inward rectification could be abolished by bath application of 1-2mM Cs which had little effect on the outward rectification but had no effect on the time-dependent inward rectification. Carbachol (10-60mM), 5-AP (2mM) and Ca-channel blockers Co or Cd, had no effect on the non-linearity of the membrane in the oscillatory voltage level. In control ringer solution both inward and outward ions almost completely abolished the subthreshold oscillatory activity. The results suggest that in addition to a persistent inward Na-current and a Q-like current, a voltage-dependent outward current plays a major role in the generation of the Rhythmic subthreshold oscillatory activity in entorhinal cortex layer II stellate cells. (Supported by MRC Canada).


Serotonin (5-HT) is localized to a subset of gonadotrophs in the mammalian anterior pituitary gland (Payette et al., 1985) and elicits ACTH release from dispersed anterior pituitary cells (Spinelli and Negro-Vilar, 1983). In the present experiments the effects of 5-HT were examined on a homogenous population of corticotrophs (AT-T-20/D16V cells). 5-HT was applied by pressure ejection onto single AT-T-20 cells plated on glass coverslips and maintained in HEPES-buffered solution. 5-HT activated these cells by producing a transient depolarization which could be superimposed upon spontaneous membrane oscillations. The amplitude and duration of the response to 5-HT depended on the pulse duration and did not desensitize. The depolarization was attributed to an increased conductance and exhibited a complex voltage dependence. These results demonstrate a direct excitatory effect of 5-HT on AT-T-20 cells which may initiate or regulate the release of ACTH. (Supported by NIH grants NS25973, NSP9010044).


The actions of serotonin (5-HT) were examined on AT-T-20 mouse corticotropic cells. Individual cells were loaded with fura-2/AM and examined for changes in extracellular calcium concentration using ratio fluorescence of fura-2. 5-HT produced a concentration-dependent increase in (Ca)j in microelectrode studies. The response was repeatable, lasted for minutes, and was characterized by a transient elevation either with or without a plateau. At the peak of the transient calcium elevation, some cells exhibited additional, rapid, repetitive calcium transients which were also observed in the absence of extracellular calcium. Twenty percent of the cells in culture exhibited rhythmic spontaneous calcium oscillations of (Ca)j, and consistent with 5-HT-induced rhythmic oscillations of (Ca)j in quiescent cells. 5-HT responses could be superimposed on calcium oscillations. The results demonstrate that 5-HT increases (Ca)j and may modulate the release of ACTH from corticotropic cells. (Supported by NIH grants NS27319 and NS25973).
LIMITED CORRELATION BETWEEN SYMPATHETIC NEURONE SUBTYPE AND PEPTIDE CONTENT IN GUINEA PIG PREVERTEBRAL GANGLIA. E.M. McLachlan, J.R. Keast and R.L. Queensland, Qld 4072, AUSTRALIA.

Guinea pig sympathetic neurones can be classified as phasic, tonic or long afterhyperpolarizing (LAH), by their discharge pattern following a depolarizing current step. These patterns result from the expression of different populations of voltage- and calcium-dependent K+ channels. Each neurone type may express a characteristic pattern of synaptic input. The proportions of each type in prevertebral ganglia of young guinea pigs varied rostrocaudally from the coeliac (CG) to the distal inferior mesenteric ganglion (IMG), with the highest proportions of CG LAH, and LAH cells being virtually absent in the IMG. The proportion of phasic neurones was similar in all ganglia.

Calcium-treated ganglia were stained immunohistochemically using antibodies to somatostatin (SOM) and neuropeptide Y (NPY). The proportions of NPY+ (30-40%) and SOM-NPY+ (30-40%) cells varied little between ganglia, although the proportion of SOM+ cells increased rostrally (from 15 to 80%) and LAH cells being virtually absent in the IMG. There was no correlation between peptide content and neurone type in the IMG (n=14).

Intracellular analysis of biophysical membrane properties that may influence transmission in the chick ciliary ganglion, intracellular recordings were obtained from prevertebral ciliary nerve terminals (n=69) and postganglionic neurones (n=48). Under current clamp conditions, depolarizing current pulses of 1-3 ms duration applied to prevertebral ciliary cells more usually elicited a single action potential (amplitude = +4.9±1.2 mV, duration = 1.00±0.03 ms), whilst ciliary neurones fired either single or multiple spikes (amplitude = +8.9±3.1 mV, duration = 1.32±0.06 ms). These neurones are characterized by a current step of lower intensity (0.2-0.8 nA), a 1 μM TTX totally abolished spike generation in ciliary and cell bodies, suggesting that a fast inward Na+ current is responsible for the generation of the action potential. Both application of Ba2+ (5 mM), TEA (10 mM) and 4-AP (1 mM), which are known to block M-current, the delayed rectifier potassium current and AHP, respectively, prolonged spike generation and increased neuronal excitability by decreasing the threshold for action potential generation when compared to similar current pulses delivered to these cells in normal recording medium. The results are a first step towards identifying those ion channels which are involved in transmission in this parasympathetic ganglion. Supported by NIH grant EY06564 to VAC.

EXCITATORY AMINO ACIDS: PHARMACOLOGY VI


Evidence for a neuronal localization of SDHA (Glut) transport including enrichment in "synaptosomal membrane fractions" and the decreases in uptake observed after neuronal lesions. Autoradiograms suggest a glial localization. Two subtypes of SDHA-L-[3H]-Glut transport can be differentiated by the selective inhibitors L-α-aminoacid (a-AAD) and dihydrokainate (DHK). Uptake in cerebellum is inhibited by AAD and insensitive to DHK. The opposite pattern is observed in forebrain regions (Brain Res., 544(1991)196). In these studies, the localization of the subtypes of transporters was investigated. Subcellular fractionation of these tissues using discontinuous sucrose density gradients demonstrated that both subtypes were at least 4-fold enriched in the synaptosomal membranes fraction. Little or no transport activity was observed in myelin or mitochondrial fractions. Previous studies have demonstrated that both crude synaptosomal membranes (P2) and synaptosomes can contain significant Glut transport (Brain Res., 101(1976)134). As β-alanine is a more potent inhibitor of GABA uptake into glia than of Glut uptake measured at 50 μM (see accompanying abs. by Dowd et al.). These subtypes of SDHA (Glut) transport can be differentiated by competitive inhibitors including d-AP (2,3-dihydroxy-6-nitrocinnamic acid), TTX (tetrodotoxin) and Cytos (cytosine). These compounds included EAA analogs that were not previously thought to interact with transport including (numbers in parentheses are IC50's in μM for control then K+ sensitive): L-glutamate (5; 12), L-aspartate (100; 23), quisqualate (66; 95), D-glutamate (236; 57), and trans (dicarboxyl)-2,4-methanoglutamate (25; 5). Compounds that inhibited less than 65% at 1 mM included: ibotenate, quisoline and AMPA. Cerebellar and hippocampal slices were similarly depolarized by 30 mM K+. Following depolarization, the uptake of L-[3H]-Glut was measured in crude synaptosomal membranes. Compared to control (incubation in normal K+), the Vmax for uptake increased 2.1-fold by depolarization in cerebellar slices (DHK-insensitive). There was no change in hippocampus (DHK-sensitive). These data add further support for the differential localization of transporters and these subtypes may be independently regulated. The effect of K+ may have implications for the vulnerability of different brain regions to excitotoxicity during ischemic insults.

The conversion of glutamine (GLU) to glutamate (GLU) is catalyzed by PAG. PAG is under physiological regulation by end product inhibition due to GLU and ammonia and is activated by both phosphate and calcium. The pharmacological specificity of the binding site on PAG for GLU-induced allosteric inhibition of PAG activity was probed. In addition, the noncompetitive N-methyl-D-aspartate (NMDA) receptor blocker (+)-MK-801 was also examined.

PAG activity was measured in crude synaptosomal (P) preparations by determining GLU formation after a 15 minute incubation with 86RbCI. The GLU formed was measured by HPLC. PAG activity was measured in the cortex from 6 month old P344 rats (n=50) and 100 day old Sprague-Dawley (SD) rats (n=5).

PAG activity (expressed as percent of control) was significantly inhibited by 5 mM of the following drugs: kainic acid (44±2%), NMDA (60±5%), quisqualic acid (44±2%), kynurenic acid (38±4%), AP5 (30±5%) and cis-pipendine dicarbocyclic acid (53±8%). Interestingly, (+)-MK-801 (5mM) stimulated PAG activity (209±10%) in P344 rats. (+)-MK-801 (5mM) was tested in SD rats and was also found to stimulate PAG activity (158±11%). Ketamine (5mM) stimulated PAG activity (137±19%) whereas (-)-MK-801 (5mM) had no effect on PAG activity. (+)-MK-801 (5mM) was additive with the stimulating effects of calcium (1mM) on PAG activity and synergistically potentiated the effects of substrate and substrate (GLU) synthesis by PAG. The results show that GLU analogs can inhibit PAG activity and demonstrate that high concentrations of (+)-MK-801 can activate PAG activity in a stereospecific manner. **

536.5
NMDA ANTAGONISTS BLOCK C-FOS EXPRESSION DURING PPOID WITHDRAWAL. C.E. Inturrisi, M. Brodsky* and K. Rasmussen, Dept. of Pharmacology, Cornell U. Med. College, New York, NY and Lilly Research Labs, Indianapolis, IN.

Naltrexone (NTX)-precipitated withdrawal in morphine dependent rats results in the induction of the proto-proto oncogene, c-fos. (Hayward et al.), Brain Res. 255, 236, 1983 and NMDA antagonists (MK-801 (MK) and LY-27461) (LY) block the behavioral signs of withdrawal (Rasmussen et al., Eur. J. Pharmacol., in press). In morphine dependent rats c-fos mRNA levels, as measured by a quantitative solution hybridization assay, are increased 2-4 fold at 1 hour after NTX withdrawal. In focus cerebellum (L), amygdala (AD), nucleus accumbens (NA), frontal cortex (FC) and hippocampus (HC) (but not in striatum or spinal cord) Pretreatment with MK-801 (5mg/kg ip) or LY (100 mg/kg ip) prior to NTX reduced by 60% to 90% the NTX withdrawal-induced increase in c-fos mRNA in AD, NA, HC and LC but not in FC. CNS region effects were noted: LY was a more effective blocker of c-fos induction in NA, while MK was more effective in LC and HC. These results demonstrate that competitive (LY) and noncompetitive (MK) NMDA antagonists can block both the behavioral manifestations of withdrawal and c-fos induction in those brain regions associated with opiate effects and withdrawal. Supported in part by NIDA Grant DA-01457 (CEI).
356.11

L-α-AMINOACIDIC REDUCES KYNURENIC ACID PRODUCTION IN THE RAT HIPPOCampus: A MICRODIALYSIS STUDY IN FREELY MOVING RATS. R. Schwarz and R.-D. Wu. Maryland Psychiatric Research Center, Baltimore, MD 21224. Kynurenine acid (KYNA) is a broad-spectrum antagonist of excitatory amino acid (EAA) receptors in mammalian brain. Neuronal activity can influence KYNA synthesis in the hippocampus and in vivo, and certain EAs have been shown to regulate KYNA function in brain slices (Synapse. 1989; 5: 328-31, 1989). The effect of the non-competitive and endogenous EAA in vivo. L-α-aminoacidic (L-αAA), was now studied by microdialysis in the rat hippocampus in vivo. αAA, administered through the dialysis probe, caused a decrease of extracellular KYNA concentration in the presence of kynurenine (500 μM), reaching 50% of control levels within 2 h after deactivation of the αAA perfusion. 500 μM αAA also lowered endogenous extracellular KYNA levels by 30%. Neuronal dejection by glutamatic acid lesions did not interfere with the αAA effect. Co-perfusion of 50 μM veratridine (which by itself potently decreases extracellular KYNA) with 500 μM αAA did not result in an additive KYNA reduction. Moreover, the αAA-induced effect was not blocked by tetrodotoxin (5 μM). These data suggest that αAA exerts its inhibitory effect through direct interaction with KYNA-producing glial cells. αAA may be an endogenous modulator of KYNA synthesis in the brain and may play a role in the function or dysfunction of cerebral EAA receptors. Supported by USPHS grant NS 16102.

356.12

MODULATION OF D-(+)-ASPARTATE RELEASE BY KAINIC ACID IS NOT MEDIATED BY AUTORECEPTOR ACTIVATION. A.M. Palmer and C.T. Reiter. Department of Psychiatry and Pharmacology, University of Pittsburgh Medical Center, 3811 O'Hara Street, Pittsburgh, PA 15213. This study addresses the hypothesis that kainic acid receptors are present on excitatory amino acid (EAA) nerve terminals. Slices of rat cerebral cortex were precipitated in physiological conditions containing kainic acid, which was collected (16 mM/l) and collection of twelve 0.5 min fractions began at t = 30 min. Kainic acid (KA, 0.1 and 1 mM at = 15-36 min) increased baseline counts in a dose-dependent fashion in both the presence and absence of 10 μM TTX. These data are not consistent with the hypothesis that kainic acid acts directly on EAA receptors.
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EXCITATORY AMINO ACIDS: PHARMACOLOGY VI
THURSDAY PM

536.15

COMPARISON OF CARBACHOL- AND GLUTAMATE-STIMULATED PHOSPHORYLATION OF RAT HIPPOCAMPAL MEMBRANE PROTEINS. L.M. Shaffer, M.A.M. Sagher and J.A. Donahue. Departments of Neurology and Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH 43669.

Carbachol and glutamatergic agonists stimulate the phosphorylation of phosphoserine, 4.5 biphosphate. These effects are differentially inhibited by active phorbol esters. Since phorbol esters stimulate the activity of PKC, it was of interest to examine changes in the phosphorylation of membrane proteins correlated with manipulation of the cholinergic and glutamatergic systems. In hippocampal slices of adult rat hippocampus, slices with 2.0 mM carbachol, no differences in the [32P]ATP phosphorylation of rat proteins were observed. However, incubation with glutamate, ibotenic acid and NMDA (10 mM) resulted in altered phosphorylation of membrane proteins. These changes in phosphorylation were identified by immunoblot analysis to be B-50/GAP-43. In contrast, increased phosphorylation in response to carbachol, glutamate and ibotenic acid was observed in hippocampal slices from 4 day old pups. Supported by grants from the Ohio Department of Aging and NIH (NS 23596).

536.17

GABAERGIC MEDIATION OF PHENCYCLIDINE-INDUCED CHANGES IN STRIATAL NEUROPEPTIDE Y LEVELS IN THE RAT BRAIN. L.P. Midgley, L.G. Bush*, J.W. Gibb and Glen R. Hanson. Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Alterations in neuropeptide Y (NPY) tissue content in CRS or extracellular fluid have been found in several psychiatric disturbances and neurodegenerative diseases. Phencyclidine-HCl (PCP), a psychotomimetic drug of abuse, interacts with a number of neurotransmitter systems and has been identified as an antagonist at the NMDA receptor. We previously reported that administration of PCP or the non-opioid NMDA antagonist, MK-801, significantly reduced (by 30-50%) striatal NPY levels in the rat brain. In the present study, we observed that administration of the GABA agonist, muscimol, and carbachol-gluconic acid (ADDA) and MDL 71,754 (gamma-vinyl-GABA, GVG), had no effect on striatal NPY levels. Conversely, the GABA antagonist, bicuculline, significantly reduced striatal NPY levels by approximately 20%. Pretreatment of rats with ADDA or MDL 71,754 completely blocked the effects of PCP and MSO-81 on striatal NPY levels. These data suggest that GABAergic receptors mediate PCP- and MSO-81-induced changes in striatal NPY systems and contribute to interactions between NMDA and NPY systems. (MDL 71,754 was graciously provided by Marion Merril Dow Inc., and the research was supported by USPHS grants DA 00869 and DA 04222).

536.18

EXTRACELLULAR FLUID GLUTAMATE CONCENTRATION IN THE PARAVENTRICULAR NUCLEUS: THE EFFECT OF POTASSIUM CHLORIDE DEPOLARIZATION AND GABAERGIC STIMULATION. M. Tristan-Morales*, W.W. Morgan and J.R. Haywood. Departments of Pharmacology and Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284.

The goal of these studies was to measure neuronal glutamate concentrations in the paraventricular nucleus (PNV) as an estimate of glutamate release using microdialysis. In chloral hydrate anesthetized rats, a 0.9 mm concentric design dialysis probe was inserted in the PNV through a previously placed guide cannula. Artificial cerebrospinal fluid was infused at a rate of 0.5 μl/min, and samples were taken at 30 min intervals. Glutamate was measured in the effluent using HPLC with electrochemical detection. Recovery of glutamate was determined at 3.6%. Potassium chloride depolarization increased extracellular glutamate concentrations from 26.3±6.3 pg/μl during the first collection period and 131.9±8.5 pg/μl during the second period. Administration of the GABA uptake inhibitor, reversepeptide base (100μM), reduced glutamate baseline concentration from 31.6±4.2 pg/μl to 17.6±2.4 pg/μl. These findings suggest that glutamate is neuronally released in the PNV. Further, the release of glutamate can be inhibited by increased release in extracellular concentrations of GABA. (Supported by HL 36080 and HL 32977)

536.19

NEUROPROTECTIVE PHARMACOLOGY AND ONTOGENY OF QUINOLINATE INDUCED INJURY IN DEVELOPING BRAIN. W.H. Trachten, L.W. McDonald and M.J. Johnson. The Kennedy Research Institute, Johns Hopkins University School of Medicine, Department of Neurology, Baltimore, MD 21205.

We evaluated the pharmacology and ontogeny of quinolinate induced injury in the immature rat brain. The dose response of quinolinate induced injury was determined in the PND 7 rat pup by unilateral microinjection of 0.5 μl of 4 doses of quinolinate (50-400 nmol, n = 6) directed into the anterior striatum. The severity of brain injury was quantified on PND 12 by comparison of the wet weights of the hemisphere ipsilateral (I) and contralateral (C) to the injection, and expressed as % hemispheric weight loss. Complete protection was provided by MK-801, phencyclidine-HCl (PCP), a psychotropic drug of abuse, interacts with a number of neurotransmitter systems and has been identified as an antagonist at the NMDA receptor. We previously reported that administration of PCP or the non-opioid NMDA antagonist, MK-801, significantly reduced (by 30-50%) striatal NPY levels in the rat brain. In the present study, we observed that administration of the GABA agonist, muscimol, and carbachol-gluconic acid (ADDA) and MDL 71,754 (gamma-vinyl-GABA, GVG), had no effect on striatal NPY levels. Conversely, the GABA antagonist, bicuculline, significantly reduced striatal NPY levels by approximately 20%. Pretreatment of rats with ADDA or MDL 71,754 completely blocked the effects of PCP and MSO-81 on striatal NPY levels. These data suggest that GABAergic receptors mediate PCP- and MSO-81-induced changes in striatal NPY systems and contribute to interactions between NMDA and NPY systems. (MDL 71,754 was graciously provided by Marion Merril Dow Inc., and the research was supported by USPHS grants DA 00869 and DA 04222).

536.20

EFFECT OF IBOTENIC ACID LESIONS ON NMDA, DOPAMINE D1- AND D2-MEDIATED CHANGES IN STRIATAL NEUROTENSIN SYSTEMS. N. Singh, L. Midgley, J. Wagstaff*, L. Bush*, J. Gibb and G. Hanson. Dept. Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT 84112.

N-methyl-D-aspartate (NMDA) receptors mediate dopamine D1-, but not D2-mediated changes in striatal neuropeptides (NPY) levels. To elucidate further the role of NMDA receptors in regulating NT systems, we measured changes in striatal NT levels following local NMDA lesions. Remaining NT levels were determined by RIA after treatment with NMDA, SKF38393 (D1 agonist) and sulpiride (D2 antagonist). Substance P (SP) levels were used to determine the extent of lesioning. NMDA lesions were found to reduce striatal NT levels significantly. Animals with >60% NT depletion were treated with 3 doses of NMDA (10 mg/kg/dose), SKF38393 (20 mg/kg/dose) and sulpiride (80 mg/kg/dose) 6 hr apart and sacrificed 16 hr later. In animals with >60% NT depletion, NT levels were reduced by 26-38% dose and did not block sulpiride-induced increases in striatal NT when compared to unoperated controls. In preliminary studies, IBO lesions also appeared to block SKF38339-induced increases in striatal NT, but did not block sulpiride-induced increases in striatal NT when compared to unoperated controls. In these studies, sulpiride reduced striatal NT levels significantly and did not block sulpiride-induced increases in striatal NT when compared to unoperated controls. In preliminary studies, IBO lesions also appeared to block SKF38339-induced increases in striatal NT, but did not block sulpiride-induced increases in striatal NT when compared to unoperated controls. In these studies, sulpiride reduced striatal NT levels significantly and did not block sulpiride-induced increases in striatal NT when compared to unoperated controls.
STUDIES ON THE MECHANISM OF INTERACTION OF THE VOLATILE ANESTHETIC HALOTHANE WITH GABA-A RECEPTORS. R. Longoni and R.G. Olsen. Dept. of Pharmacology, School of Medicine, University of California, Los Angeles, CA 90024.

Volatile anesthetics enhance GABA-A receptor-chloride channel function in some neurons. The effects of halothane on GABA-A receptor-related chloride flux were evaluated in brain slices. Rat cortical slices were prepared according to the method of Yang and Olsen (1987). [3H]4'Cl-diazepam and [3H]PK11195 bound to the GABA-A receptors. The affinity of [3H]4'Cl-diazepam was high (Ki 2-20 nM) and [3H]PK11195 was lower (Ki 5-100 nM). Simultaneous addition of halothane at clinically relevant concentrations of 0.5-1.7 μM augmented and prolonged the muscimol-dependent [3H]4'Cl efflux without affecting the basal flux. In fact, in a Ca2+ free Ringer's solution, no halothane effect could be observed, while subsequent enhancement of muscimol function was not impaired. GABA-mediated inhibition by halothane may be more complicated than direct modulation of GABA-A receptors by other CNS depressants. It may contribute to our understanding of the role of GABA neurotransmission in general anesthesia.

PIRACETAM BLOCK OF GABA CURRENT BY TWO DIFFERENT MECHANISMS. Kung-Yee Yoon, D. F. Covey, and Steven M. Ratham. Department of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We examined the effect of the picrotoxin (PTX) receptor antagonist, d-isopropyl-methyl-γ-butyrolactone (dIMGBL), and PTX on GABA induced chloride currents in dissociated rat hippocampal neurons. When applied by U-tube, PTX (40 μM) decreased the peak GABA (10 μM) current by 36.6 ± 3.9%. The amount of PTX block increased to 73.6 ± 2.8% with continued application with a time constant of 2.1 ± 0.2 sec. The amount of early and late depression by picrotoxin was not dependent on GABA concentration in the range of 1 to 30 μM but it was dependent on picrotoxin concentration (10, 40μM). The time constant for the delayed block decreased with increasing GABA concentration (20 sec at 1 μM, 1 sec at 30 μM) suggesting further that this PTX effect is use dependent. In a separate set of experiments the picrotoxin receptor antagonist dIMGBL (5μM) almost completely abolished the delayed block (74.4 ± 2.4% to 38.5 ± 10.1%, n=7) while it had no effect on the early current depression. With brief application of 1 μM GABA, we saw no use dependent PTX block and no reduction of block by dIMGBL. Our observations suggest that the PTX block of GABA current is mediated at different sites producing a fast and slow (use dependent) block. dIMGBL appear to selectively antagonize the use dependent block.

Supported by NS 14834.

THE MUSCLE RELAXANT EFFECT OF FLUPRIPINE - INDICATIONS FROM ELECTROPHYSIOLOGICAL STUDIES. M. Wienrich and I. Szelenyi. Dept. Neurobiology, Battelle Europe and ASTA-Pharma AG*, D-6000 Frankfurt am Main, Germany.

Flupiripine (F) is a centrally acting, non-opioid analgesic. Analgesia caused by F is accompanied by a muscle relaxant activity. In order to elucidate the mechanism of this effect, we studied the interaction of F with GABA. We used primary dissociated cultures derived from whole embryonic rat brain and performed whole-cell recordings in the current-clamp mode. Due to our recording conditions, GABA induced reversible, concentration-dependent membrane depolarizations (EC50 1.5±10M) which were antagonized by bicuculline, indicating the presence of a GABA-A receptor. F also induced reversible, concentration-dependent membrane depolarizations (EC50 1.5±10M) which were also antagonized by bicuculline. At 1μM GABA induced a maximal effect. At the same concentration F, however, had no effect. Moreover, F at a threshold concentration of 3μM potentiated the GABA effect, causing the concentration response curve for GABA to shift to the left (EC50 1.5±10M). This indicates a possible over-additive interaction between the two compounds. We conclude that F has GABA-A agonistic properties.
537.7

ABECARNIL: A β-CARBOLINE ANXIOLYTIC SHOWING A SELECTIVE ACTION AT CENTRAL BENZODIAZEPINE RECEPTORS.


The β-carboline abecarnil shows anxiolytic and anticonvulsant effects in animal models at lower levels of benzodiazepine receptor occupation than diazepam, but does not exert muscle relaxant or ataxic effects. (Stephens et al, JPET 253: 334, 1990; Turski et al, JPET 253: 344, 1990). These observations suggest some selectivity of modulation of benzodiazepine receptors. Consistent with this hypothesis, abecarnil in vitro showed a 15 fold lower affinity for 3H-homotratzazepam binding sites in spinal cord than in cortex (IC50 50±15 μM), whereas diazepam showed a similar affinity for the two sites (250±80 μM). In cerebellum, abecarnil was able to displace binding of 3H-Ro 15-4513 from both its binding sites (IC50 2 and 560 nM), whereas diazepam and other benzodiazepines displaced maximally 60% of Ro 15-4513 binding (IC50 20 ± +10000 nM). Electrophysiological studies in frog oocytes expressing defined combinations of benzodiazepine receptor subunits confirmed a differentiation between abecarnil and benzodiazepines. These findings are consistent with abecarnil interacting with an overlapping but different subpopulation of receptors from benzodiazepines.

537.9

QUANTITATIVE AUTORADIOGRAPHIC CHARACTERIZATION OF THE BINDING OF 3H-NO 328 (TIAGabINE) TO THE GABA UPTAKE CARRIER. P.D. Subramaniam and K.E. Anderson. Novo Nordisk A/S, CNS Division, DK-2860 Soeborg, Denmark.

Gaba (Gamma aminobutyric acid) is a major inhibitory neurotransmitter in the CNS. A decrease in GABA-ergic neurotransmission is believed to be involved in the neurological conditions, including epilepsy. NO 328 (TiaGabine) is a potent and selective inhibitor of both GABA uptake in vitro (IC50 67 nM) and DMC and PFT-induced convulsions in mice and rats. The present report examines the autoradiographic localization of the GABA uptake carrier in rat brain using 3H-NO 328. 3H-NO 328 binding was regionally distributed in the rat brain, with the highest level of binding present in the prefrontal cortex, somatosensory cortex, superior colliculus, substantia nigra pars compacta, dorsal raphe, amygdaloid nucleus, CA and CA of the hippocampus, and molecular layer of the cerebellum. Scatchard analysis serial sections were linear with dissociation constant values ranging from 10 nM (frontopontal cortex, motor area) to 165 nM (globus pallidus) and linear ranging from 454 pmol/mg protein (substantia nigra pars reticulata) to 28 pmol/mg protein (corpus colosum).

537.11


We have reported that high affinity temperature-dependent cellular uptake of triiodothyronine (T3) into HepG2 cells is inhibited by um diazepam and peripheral ligand ROS 4684 (Soc for NS 1990, abstr #36.19). This inhibition is due to toxic and is reversible with washing. We assayed the dose-response of 25 benzodiazepines (BZs) and determined apparent IC50 to illustrate structure for inhibition. ASSAY: Confluent HepG2 cells in multiwell plates were incubated 3 hr prior to assay with serum-free media. Wells +/- drug and 40 pM [3H]-T3 incubated 1 hr at 22 or 4 C, then cells were washed on ice. The NaOH hydrolysate was gamma counted. T3 uptake at 22 C: -4 C uptake = 80% of total 22 C uptake. RESULTS: At R1, Cl>Br; at R2, Na; at R3, NO3; at R4, CI; at R5, OH; at R6, alky! groups > H; on imidazole of 1,2 annelated BZs, CI>OH; the nonphenyl pyrimid and the carbonyl A at positions 1,4 are essential for potency. Most potent drugs were lorazepam, triazolam, and prazepam and least were flumazenil and RO5 3663. Lorazepam and triazolam showed no effect on T3 binding to isolated rat liver nuclei, indicating that the interaction is not at the nuclear receptor. T3 did not affect ROS 4584 binding to rat liver homogenate, indicating that it does not share binding sites with this peripheral BZ ligand. Study supported by NIH grant K1 1R01DA04566 to Dr. Kraig. P. Gorter of HoffmanLaRoche generously supplied benzodiazepines.

537.12

DEHYDROEPIANDROSTROSTERONE SULFATE: A NEUROSTEROID THAT BLOCKS THE GABA A RECEPTOR NONCOMPETITIVELY. C.E. Spiga. Laboratory of Molecular Neurobiology, NIDA/ARC, P&D, Box 5180, Baltimore, MD 21224.

Dehydroepiandrosterone sulfate (DHEAS) was tested at GABA receptors on neurons cultured from the ventral mesencephalon of fetal rats. The currents were measured under whole cell voltage clamp. DHEAS blocked peak currents induced by GABA noncompetitively with dissociation constant of 4.5 μM and a half-activation potential of 22 mV. In addition, DHEAS caused a pronounced, double exponential decay in the conductance. Analysis showed that this decay was not due to any of the following: (1) accelerated desensitization of the receptor, which showed single exponential decay, (2) voltage dependent occlusion of the ion channel by DHEAS, or (3) binding of DHEAS to the activated state of the receptor. DHEAS also decreased the time constant for the decay of large, but not small, inhibitory postsynaptic currents. This observation is attributed to either diminished release of GABA or accelerated reuptake.

Certain naturally occurring steroids that exist as trace metabolites of steroid hormones have been found to enhance GABAergic receptor function and bind. One of the most potent of these in vitro studies is a THDOC (5α-pregnane-3α,21-diol-20-one), THDOC, presumably acting via GABAergic mechanisms, possesses anxiolytic and sedative, hypnotic, anticonvulsant and anxiolytic properties. In this study, Southern California, Los Angeles, CA 90033, we have found to enhance GABAergic receptor function and bind. This study, the effects of THDOC on CRF neurons in the locus ceruleus, we have examined the acute effects of THDOC on CRF neurons in several brain regions and on plasma corticosterone concentrations. In addition, we concomitantly examined the actions of the synthetic glucocorticoid, dexamethasone, and its purportedly active metabolite, 6β-dexamethasone, on these same measures of CRF activity. A non-glucocorticoid mechanism has been previously demonstrated in the defensive burying paradigm. The anxiogenic effects of the steroid treatment were evidenced in a doubling of the weight of the ventral prostate gland in TP-treated males. The effect of the BARA/BZD receptor system was investigated using [3H] uptake in cortical synaptosomes. Whereas TP treatment did not affect the maximal response to GABA, a significant decrease in the Emax for GABA-stimulated Cl- influx was observed. This pattern of results has been previously demonstrated following discontinuation from chronic treatment with BDZs, and is consistent with an upregulation in the function of the BARA/BZD receptor complex. In our studies, spinal capsase were found empty upon sacrifice, suggesting that the rats were in a withdrawal phase. Current experiments will more precisely characterize changes in behavior and neural function during TP exposure and following steroid withdrawal.

THDOC  (5-40 mg/kg) significantly increased the number of light/dark transitions. Unlike alprazolam, THDOC failed to decrease CRF concentrations in the locus ceruleus. While THDOC may plausibly act endogenously to reduce stress-induced endocrine and behavioral responses that are likely mediated in part by CRF neurons, these preliminary findings suggest that THDOC does not identically mimic the actions of alprazolam, another drug which potentiates GABAergic activity.

ANXIOLYTIC ACTIVITY OF THE PROGESTERONE METABOLITE 5α-PREGNAN-3α-OH-20-ONE. S. Wieland, N.C. Lan, S. Mirasidedeghi* and K.W. Goo. Dep. of Molecular Pharmacology and Toxicology, Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033. 3α-hydroxylated progesterone steroids have been shown to possess anesthetic, hypnotic, anticonvulsant and anxiolytic properties. In this study, metabolites of progesterone and deoxycorticosterone, 5α-pregnan-3α-ol-20-one (5α-pregnan-3α-OH-DHP) and 5α-pregnan-3α-ol-21-diol-20-one (5α-THDOC), respectively, were tested for anxiolytic effects in N.I.H. Swiss Webster mice using the light/dark transition, open-field and lick-suppression tests. Similar to the benzodiazepine, diazepam (5α-OH-DHP), 5α-pregnan-3α-OH-DHP (10 mg/kg) blocked diazepam’s (1.0 mg/kg) anxiolytic effects, but did not have any effect against 3α-OH-DHP (20 mg/kg). The data indicate that the pregnane steroids produce their anxiolytic effects through a separate mechanism than the BZs. 3α-OH-DHP (20 mg/kg), 5α-THDOC (20 mg/kg) and diazepam (1.0 mg/kg) increased activity in an open-field test. 3α-OH-DHP had no effect in the open-field test. Furthermore, 3α-OH-DHP produced a 235% increase in punished responding in a lick-suppression test. These results demonstrate that the endogenous pregnane steroids possess anxiolytic effects that may be clinically relevant. (Supported by grants from CoCenyes, Inc. and NS 24645 and NS 25866).

TEMPERATURE DEPENDENCE AND STEROID MODULATION OF [3H]FLUNITRIZEPAM BINDING TO RAT BRAIN MEMBRANES. R.J. Prince and A.M. Simmonds. School of Pharmacy, University of London, London WC1N 1AX. The state of fluidity of lipid bilayers is dependent upon both the steroids present, notably cholesterol, and temperature. We have examined the effect of temperature upon [3H]flunitrazepam (FNZ) binding to the GABAa/benzodiazepine receptor and also upon the modulation of this receptor by the pregnane steroid alprazolam. The affinity of FNZ was measured at temperatures in the range 0-47°C using Scatchard plotting and by rapid filtration in the absence and presence of 100μM alprazolam. Increasing temperature resulted in a decrease in affinity. A plot of reciprocal absolute-temperature against -log[(Kd)] showed a linear relationship in both cases. This is predicted by the van’t Hoff equation (lnKp = -H/RT + A/R) and indicates that affinity changes may be due to thermodynamic considerations only. Linear regression yields values of -30.23 and -34.04 kJ/mol for ΔH and 53.51 and 47.88 kJ/mol for ΔS in the absence and presence of steroid respectively. The derived values for ΔH are not significantly different at a confidence level of 95% indicating that steroid potentiation may be a temperature independent phenomenon.

EFFECTS OF CHRONIC ANABOLIC/ANDROGENIC STEROID EXPOSURE ON INDICES OF FUNCTION OF THE GABA/BZD/APAP RECEPTOR COMPLEX. Daniel Bittner, Robert J. Shih* and Carol R. Kellig. University of Rochester, Department of Psychology, River Campus, Rochester, NY 14627.

The growing use of anabolic/androgenic steroids by athletes is of great concern to the medical and scientific communities. Although much research has focused on the mechanisms underlying steroid-mediated anabolic effects, little is known about the neural mechanisms that mediate the adverse effects on mental health. In addition to the classic genomic mechanism of steroid action, the modulation of neural excitability by 3α-hydroxylated pregnane steroids via effects on GABA/BZD receptors has been demonstrated. Reduced modulates of testosterone potentiate GABA-mediated function in a manner similar to that observed for reduced pregnane steroids. Since anabolic/androgenic steroid metabolism is a significant issue at the GABA/BZD receptor complex, it was hypothesized that behavioral and biochemical indices of the GABA/BZD receptor system would be affected by chronic treatment, and subsequent withdrawal from testosterone-proprionate (TP) exposure.

In one experiment, 4 weeks following the implantation of a TP-filled silastic capsule (30 mm), male rats displayed increased anxiety as shown by an increase in the duration of burying behavior in the defensive burying paradigm. The anxiogenic efficacy of the steroid treatment was evidenced in a doubling of the weight of the ventral prostate gland in TP-treated males. The effect of the BARA/BZD receptor system was investigated using [3H] uptake in cortical synaptosomes. Whereas TP treatment did not affect the maximal response to GABA, a significant decrease in the Emax for GABA-stimulated Cl- influx was observed. This pattern of results has been previously demonstrated following discontinuation from chronic treatment with BDZs, and is consistent with an upregulation in the function of the BARA/BZD receptor complex. In our studies, spinal capsase were found empty upon sacrifice, suggesting that the rats were in a withdrawal phase. Current experiments will more precisely characterize changes in behavior and neural function during TP exposure and following steroid withdrawal.

ANXIOLYTIC ACTIVITY OF THE PROGESTERONE METABOLITE 5α-PREGNAN-3α-OH-20-ONE. S. Wieland, N.C. Lan, S. Mirasidedeghi* and K.W. Goo. Dep. of Molecular Pharmacology and Toxicology, Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033. The anatomical localization of the putative steroid receptor site associated with the GABAa/BZD receptor complex was examined in rat brain by autoradiography. (Supported by a grant from CoCenyes, Inc.)

TEMPERATURE DEPENDENCE AND STEROID MODULATION OF [3H]FLUNITRIZEPAM BINDING TO RAT BRAIN MEMBRANES. R.J. Prince and A.M. Simmonds. School of Pharmacy, University of London, London WC1N 1AX. The state of fluidity of lipid bilayers is dependent upon both the steroids present, notably cholesterol, and temperature. We have examined the effect of temperature upon [3H]flunitrazepam (FNZ) binding to the GABAa/benzodiazepine receptor and also upon the modulation of this receptor by the pregnane steroid alprazolam. The affinity of FNZ was measured at temperatures in the range 0-47°C using Scatchard plotting and by rapid filtration in the absence and presence of 100μM alprazolam. Increasing temperature resulted in a decrease in affinity. A plot of reciprocal absolute-temperature against -log(Kd)] showed a linear relationship in both cases. This is predicted by the van’t Hoff equation (lnKp = -H/RT + A/R) and indicates that affinity changes may be due to thermodynamic considerations only. Linear regression yields values of -30.23 and -34.04 kJ/mol for ΔH and 53.51 and 47.88 kJ/mol for ΔS in the absence and presence of steroid respectively. The derived values for ΔH are not significantly different at a confidence level of 95% indicating that steroid potentiation may be a temperature independent phenomenon.
537.19  
**BIOCHEMICAL EVIDENCE THAT "IN VIVO" ADMINISTRATION OF K-RECAIN ELUENCES GABAERGIC TRANSMISSION IN THE RAT CEREBRAL CORTEX.**  W. Seutter*, D. A. Ghigna, G. A. Lechian, A. Canessa, E. Sanes, and G. Blicq, Dept. of Experimental Biology, Chair of Pharmacol., Univ. of Cagliari, Cagliari 09123, Italy

The effect of abecarnil (AB), a new β-carboline possessing anxiolytic and anticonvulsant properties, was evaluated "in vivo" on the function of GABA receptor complexes. The intraperitoneal injection of AB produced in 30 min a dose-dependent (0.25-20 mg/kg) decrease of [3H]GABA binding measured "ex vivo" in the rat cerebral cortex; this effect was abolished by the administration of the benzodiazepine receptor antagonist flumazenil (5.0-15.0 μg/kg). AB, up to the nasal dose used (20 μg/kg), failed to induce ataxia and loss of righting reflex. Moreover, acute administration of AB (1 mg/kg) completely antagonized the convulsive activity and the increase of [35S]TBPS binding induced by 350 mg/kg of isoniazid as well as the increase of [3H]GABA binding induced by foot-shock stress. Consistent with the above results the "in vivo" addition of AB, mimicking the action of benzodiazepines, increased [3H]GABA binding, enhanced muscimol-stimulated [3H]GABA uptake, reduced [35S]TBPS binding and inhibited the binding of [3H]flunitrazepam. The results suggest that the enhancement induced by AB on GABAergic transmission can be detected biochemically in the membrane preparation "ex vivo".

**OPIOIDS: BEHAVIOR I**

538.1  
**ALFENTANIL DOSE-EFFECT RELATIONSHIPS FOR FOUR OPIATE EFFECTS IN THE RAT.**  M. B. Weinger, P. K. Yancey, S. S. Neush, Dept. of Anesthesiology, UCSD and VA Medical Centers, San Diego, CA 92161; Department of Neuropharmacology, Scripps Clinicos, La Jolla, CA 92039.

Besides antinociception (AN) and sedation, opiates also cause catalepsy (CT) and muscle rigidity (MR). However, MR dose-effect relationships have not been directly compared to AN or loss of righting reflex (RR). To elucidate the mechanisms and sites of action of opiate anesthesia, the endpoints of opiate effect must first be described. We, therefore, studied four relevant opiate endpoints (AN, CT, MR, and RR) using the μ-agonist alfentanil (ALF) as the spontaneously ventilating rat. Rats were randomized to receive a dose of ALF (0-500 μg/kg s.c.). For AN and CT, 49 rats were studied for 120 min after ALF. CT was measured from the time the rat’s forelimb was placed on a 10-cm-high bar until either limb was removed. The nociceptive stimulus was tail immersion in 55°C water. AN latency was measured from tail immersion until purposeful tail movement (10-sec cutoff). For RR, 40 rats were studied for 120 min. MR was absent when a rat could not right itself in 15 sec. For each effect, data were converted to quantal responses by assigning any value >4 times baseline as a positive response. Data were converted to probit-log dose response curves, and slopes and ED50’s were analyzed.

Slopes between the four dose-effect curves were not significantly different. The ED50 for RR differed significantly from AN and MR, and the ED50 for CT differed significantly from AN. The slopes and ED50’s for MR and CT were similar, suggesting a common site of action. Catalepsy may be a useful model for spontaneous movement during opiate anesthesia. These techniques and data will help elucidate the pharmacology of opiate effects and their underlying receptor mechanisms.

538.3  
**OPIATE REGULATION OF AFFILIATIVE VOCALIZATIONS AND BEHAVIORS IN INFANT RHEUS MONKEYS.**  N.H. Kalin, S. E. Shelton* and C. T. Snowdon*, Dept. of Psychiatry and Psychology, UCLA, Los Angeles, CA 90024.

During reunion, endogenous opiate systems also appear to regulate infants' attachment behaviors. During reunion, endogenous opiate systems also appear to regulate infants’ attachment behaviors.

During reunion, endogenous opiate systems also appear to regulate infants' attachment behaviors.

538.4  
**SENSITIZATION OF OPIATE EFFECTS: PROGRESSIVE INCREASES IN FEEDING WITH REPEATED CENTRAL INJECTIONS OF DAGO BUT NOT DPDPE.**  H.A. Wise and M.B. Noel, Center for Studies in Behavioral Neurobiology, Dept. Psychol., Concordia University, Montreal, Quebec, Canada. H3G 1M8

Ventral tegmental area (VTA) injections of morphine cause locomotion and potentiate feeding; the locomotor effects increase (sensitize) with repeated testing. In order to determine whether mu or delta receptors are involved in the feeding effects and whether sensitization occurs in relation to the feeding as well as the locomotor effects, we examined the effects of repeated administration of the selective mu agonist DAGO ([D-Ala2,N-Me-Phe1,Gly-ol]-enkephalin) and the selective delta agonist DPDPE ([D-Pen2,D-Pen5]-enkephalin) in food deprived rats. Rats were implanted with stainless steel cannulae aimed at the VTA. Feeding behavior was quantified daily after 18 hours of food deprivation; food pellets were presented in 18 meal segments consisting of 50 45 mg food pellets per segment. VTA injections of DAGO and DPDPE caused dose-dependent acceleration of feeding; DAGO was 10 times more effective than DPDPE ([1-10 mg/kg sc] vs saline). Sensitization to the feeding effects of each drug was assessed by repeated injections of a low dose (0.1 mg/kg) of each drug once a day for 7 days (10 injections total) and was determined by a comparison of feeding responses on the first and last day of testing. DAGO resulted in progressively greater accelerations of feeding, while repeated DPDPE (0.5 mg/kg) injections did not. These data indicate mu but not delta receptors in the facilitation of feeding and indicate that sensitization of the potentiation of feeding, like the locomotor effects of opioids, is sensitized by repeated opioid treatment.
538.5 DIET AND OPIATE-INDUCED ALGAEASIA IN RATS. R. B. Kanarek, E. S. White and R. Marks-Kaufman*, Dept. of Psychology and School of Nutrition, Tufts Univ., Medford, MA 02155.

This series of experiments examined the effects of palatable foods on opiate-induced analgesia using a tail-flick apparatus in 42 adult male Sprague-Dawley rats. Rats were given ad lib access to Purina Chow (N=14), or given a choice of Purina Chow and either a 32% sucrose solution (N=14), or hypoglycated vegetable fat (N=14). Analgesic testing was conducted immediately prior and at 30, 60, and 90 minutes following intraperitoneal injections of morphine sulfate (0.0, 0.5, and 10 mg/kg) or subcutaneous injections of (+)-ketocyclazocine (KCR) (0.0, 0.1, 0.3 and 0.6 mg/kg). No differences in analgesic responsiveness were observed as a function of diet preceding drug injections. However, dietary variables did alter opiate-induced analgesia. Sixty minutes following the injections of 10 mg/kg morphine, animals fed sucrose or fat had significantly longer mean tail flick latencies than rats fed only Purina Chow (Purina Chow = 1.5 sec; Purina + sucrose = 4.9 sec; Purina + fat = 3.3 sec). Similarly, at 30 minutes following the injections of 0.3 mg/kg KCR, rats fed sucrose or fat had significantly longer mean tail flick latencies than their Purina Chow fed counterparts (Purina Chow = 0.7 sec; Purina + sucrose = 3.3 sec; Purina + fat = 2.7 sec). These data suggest that dietary variables interact directly with endogenous opioid systems to influence pain sensitivity.


Chronic pretreatment with amphetamine (AMPH) has been shown to enhance the effects of intra-ventral tegmental area (VTA) microinjections of morphine on locomotion (Stewart & Vezina, 1987) and food intake (Stewart & Vezina, 1990). In this experiment, highly selective mu- and k-opioid agonists, DAMGO and U-50,488H (USO), were compared for their effects on ingestive behaviors in rats pretreated with bilateral cannulae aimed at the VTA. Two groups received daily ip injections of either saline (SAL) or 3 ng/kg of d-amphetamine (AMPH) in their home cages. After 10 days of chronic treatment, the rats were challenged, in a counterbalanced order, with USO (1.0 nmol per side), DAGO (0.1 nmol per side) or saline (0.5 μl per side). 5 min after the microinjections, the rats were placed in cages and feeding and drinking, and other activities were recorded over 1 h. Tests occurred twice a week. DAMGO increased drinking by 66% in SAL-pretreated rats and by 185% in AMPH-pretreated rats. Water-intake after microinjection of saline was the same in the two chronic treatment groups. DAMGO did not increase food-intake, measured over 1 h, in either group. USO failed to affect both feeding and drinking. These results suggest that μ-opioid and dopaminergic pathways interact at the level of VTA to modulate drinking as well as other appetitive behaviors.


Fetal rats reliably express a facial wiping response to punctate tactile stimulation of the perioral area on days 20-21 of gestation (birth = 21.5 days). The wiping response is eliminated 60-s after intraoral infusion of milk. Pretreatment of fetuses with naloxone blocks the milk effect and restores the wiping response, indicating that milk activates a functional opioid system in the fetus. The κ-opioid agonist, nor-binaltorphimine HCl, also is effective in reversing the behavioral effects of milk, but the μ-opioid agonist, β-funaltrexamine HCl is not. These findings suggest that milk infusion triggers the release of endogenous opioids which bind to the κ receptor to alter fetal responsiveness to stimulation and effect global changes in fetal behavioral organization.

This research was supported by grant HD 16102 and RCDA RD 00719 to WPS.

Several species of African and South American fish are able to produce electric organ discharges (EOD) with varying levels of EOD rate that are both highly stable and individually unique. In Experiment 1, 75 fish (*E. magna* and *E. mexicana*) were recorded (5 ul) with either diluent, naloxone (2 mg/kg), morphine (10 mg/kg), a single injection containing naltrexone and morphine, or naltrexone followed 10 seconds later by morphine. Testing consisted of a 10 minute post-injection baseline period and a 30 minute post-injection test period. Morphine was found to significantly decrease (p<0.05) EOD rate relative to diluent and naloxone, which did not differ. A single injection of naloxone and morphine produced results similar to separate injections of naloxone and morphine; in both cases, naloxone antagonized the effects of morphine, suggesting that separate injections are not necessary. Experiment 2 evaluated doses of morphine (0.5, 5.0, 10.0, and 40.0 mg/kg IP), with results indicating that 40 mg/kg significantly reduced (p<0.05) the EOD rate relative to all other groups and even terminated the EOD rate completely in some subjects; 20 mg/kg significantly decreased (p<0.05) the EOD rate relative to the remaining doses, which did not differ from one another. The results suggest that *E. magna* and *E. mexicana* might serve as a new opiate assay.


Endogenous opioid systems have been implicated in spatial task learning. Polygynous meadow voles, *Microtus pennsylvanicus*, display profound differences in learning ability to learn spatial tasks. In the present study, we examined the effects of the opiate antagonist, naloxone (1 mg/kg), in spatial task learning (Morris water maze) of non-reproductive adult male and female meadow voles. Females treated with naloxone acquired the task faster than any other group, although all groups learned the task in five acquisition days. Contrary to previous studies with adult reproductive voles, no sex differences favoring males were detected in acquisition of the spatial task. Results from this study suggest that there may be minimal sex differences in learning ability to learn spatial tasks.

538.13 EFFECTS OF B-FUNALTREXAMINE AND NALOXONAZINE ON ALFENTANIL-INDUCED MUSCLE RIGIDITY AND ANTIMOCICEPTION IN THE RAT. S.S. Negus and M.B. Weinger, Dept. of Anesthesiology, UCSD and VA Medical Centers, San Diego, CA 92161.

Alfentanil is a potent and short-acting opioid agonist that is selective for mu opioid receptors. As such, mu opioid agonists, alfentanil produces such clinically useful effects as antinociception, while also producing such unwanted side effects as muscle rigidity. The purpose of the present study was to compare two agents: B-FNA, a selective and irreversible mu antagonist, whereas NXZ has been shown to antagonize alfentanil-induced rigidity and alfentanil-induced electromyographic activity from the gastrocnemius muscle, whereas antinociception was measured using the warm-water tail dip procedure. Alfentanil dose-effect curves were generated for each pretreatment characterized as a selective and long-acting antagonist of the mu antagonist. Baseline EMG activity was recorded for at least 15 minutes. Before ALF, the diaphragmatic EMG activity was recorded, and after ALF, the diaphragmatic EMG activity was again recorded. Thus, after ALF, the diaphragmatic EMG activity was analyzed by computer to determine the effects of ALF on induced increases in thoracic muscle tone contribute to the mechanism of the effect and the role of diaphragmatic


We have estimated that about 90% of autistic children are hyperactive as measured by the Conner's Parent Teachers Rating Scale (CPTRS). Here we demonstrate methods for measuring activity all indicate that acutely administered naltrexone significantly decreases the hyperactivity of autistic children. Placebo (P1,P2) or naltrexone (NaI) (0.5, 1.0, 1.5, 2 mg/kg) was administered once per week to autistic children (3-12 y.o.), and Sa were tested on the day of drug. Activity was measured in the BRC Social Proximity Test (BRCSPT) by determining the number of squares entered in a playroom and by recording the time spent by an ambulatory activity monitor (AAM). Parents rated 24th activity of their child on the CPTRS. The BRCSPT consists of three 10 min sessions, two in the presence of a familiar volunteer-one without toys and one with toys and a third with the mother. In the BRCSPT, NaI significantly decreased AAM scores in comparison with P1 (N=3, p<0.005; M3, SEM, P1=330±45, 1.0=169 ± 4, 1.5=151 ± 4, 2.0=186 ± 45). Similarly, NaI significantly decreased number of squares entered in the BRCSPT (N=, p<0.05; M±SEM, P1 = 31 ± 2, 0.5 ± 2.5 ± 1.10 ± 23 ± 1.5 ± 24 ± 2, 2.0 ± 26 ± 2, P2= 28 ± 2. In the CPTRS, NaI significantly reduced parents' ratings of hyperactivity (N=6, p<.05; M±SEM, P1=13 ± 3, 0.5 = 9 ± 2, 1.0 = 8 ± 0.5; 1.5 = 7 ± 2, 2.0 = 7 ± 4, P2 = 12 ± 4. Supported by FDA, NICHD, Du Pont (to BHH).


Muscle rigidity and respiratory depression are two undesirable side effects of opiate anesthesia. Opiate-induced increases in thoracic muscle tone contribute to impaired ventilation. However, the effect of high-dose opiates on diaphragmatic muscle tone and function has not been described. Therefore, we investigated the effects of alfentanil (ALF) on diaphragmatic electromyographic (EMG) activity in the spontaneously ventilating rat. 5 - 7 days before the experiment, a differential electrical nature of alfentanil-induced muscle rigidity and antinociception by evaluating the susceptibility of these effects to antagonism by B-funaltrexamine (B-FNA) and naltrexone (NXZ). B-FNA has been characterized as a selective and irreversible mu antagonist, whereas NXZ has been shown to antagonize alfentanil-induced rigidity and alfentanil-induced antinociception. These results indicate that mu receptors in general, and mu-1 receptors in particular, are capable of mediating both the antinociception and the muscle rigidity produced by alfentanil in the rat.


Spontaneous locomotor activity (fine and gross movements) was assessed on the day of drug. Activity was measured in the BRC Social Proximity Test (BRCSPT) by determining the number of squares bed movement by an ambulatory activity monitor (AAM). Parents rated 24th activity of their child on the CPTRS. The BRCSPT consists of three 10 min sessions, two in the presence of a familiar volunteer-one without toys and one with toys and a third with the mother. In the BRCSPT, NaI significantly decreased AAM scores in comparison with P1 (N=3, p<0.005; M3, SEM, P1=330±45, 1.0=169 ± 4, 1.5=151 ± 4, 2.0=186 ± 45). Similarly, NaI significantly decreased number of squares entered in the BRCSPT (N=, p<0.05; M±SEM, P1 = 31 ± 2, 0.5 ± 2.5 ± 1.10 ± 23 ± 1.5 ± 24 ± 2, 2.0 ± 26 ± 2, P2= 28 ± 2. In the CPTRS, NaI significantly reduced parents' ratings of hyperactivity (N=6, p<.05; M±SEM, P1=13 ± 3, 0.5 = 9 ± 2, 1.0 = 8 ± 0.5; 1.5 = 7 ± 2, 2.0 = 7 ± 4, P2 = 12 ± 4. Supported by FDA, NICHD, Du Pont (to BHH).
539.1

Faster than that of R-TISCH (k = 0.082 min⁻¹). The high binding affinity in dopamine receptors in rat striatal membranes (Kd = 0.20 nM) for R-TISCH stereoselective preference for the R(+) over the S(-) isomer. Kinetic

539.2

The anterior cingulate cortex is a key cortical component of the circuits implicated in emotion, reward and affective responses to pain. In the primate, the anterior cingulate cortex receives a dense mesocortical dopaminergic projection that terminates in both superficial and deep layers. With the molecular cloning of the genes for multiple dopamine receptors, it has now become feasible to investigate, using subtype-specific probes, the distribution and the distribution of the dopamine receptor-expressing neurons in the anterior cingulate cortex. cDNAs for the dopamine D1 and D2 receptors were isolated via PCR and subcloned into Bluescript plasmid. 35S-labeled DNA was synthesized in situs hybridization, which was done in 20 μM thick sections of anterior cingulate and prefrontal cortex from paraffin-embedded macaque brain. Both probes labeled neurons in lamina that receive dopaminergic input and the precise laminar pattern and density of labelling was distinct for the D1 and D2 receptors. Preliminary data suggest that D1 mRNA was most abundant in layer V, intermediate in layer III and detectable in layer VI. D1 mRNA was greatest in layer III and was also relatively high in layers II and V. We are now cloning cDNAs for the dopamine D1 and D2 receptors. Quantitative analysis of the anterior cingulate laminal distribution of these subtypes will be presented. Supported by NIH grants K11 DK01854, MH 45212 and AG06867.4.

539.3

TIME COURSE OF ENHANCED D1 AGONIST-INDUCED METABOLIC RESPONSES FOLLOWING ACUTE DOPAMINE DEPLETION. J.M. Trumpman and C.L. James*. Dept. of Neurology, Univ. of Virginia, Charlottesville, VA.

One key aspect of dopaminergic denervation supersensitivity is the enhanced efficacy of D1 agonists to stimulate metabolic responses: the increased density of D1 agonists to stimulate regional cerebral glucose utilization (RCGU) in the substantia nigra pars reticulata (SNR) in rats with chronic dopamine depletion provides a metabolic correlate to the heightened motor response. The present study examined the time course of D1 agonist-induced metabolic responses following either acute dopamine depletion with reserpine (5 mg/kg, ip) or chronic dopamine depletion with reserpine (1 mg/kg, ip) for 4 weeks. RCGU effects were assessed with [14C]-2-deoxyglucose autoradiography. The D1 agonist SKF 38393 (30 μg/kg) increased SNR RCGU by 18 % in naive rats. In comparison, SKF 38393 increased SNR RCGU by 38%, 68% and 64% at 6, 12 and 24 hours post-reserpine (p < 0.05). The response returned to control values at 5 days. D1 and D2 receptor number in the striatum and SNR, as measured by antagonist ligand binding, were unchanged 24 hours post-reserpine. We conclude that the metabolic effects of D1 stimulation are clearly enhanced within 12 hours of reserpine injection and that the enhanced effects are not mediated by an increase in D1 receptor number. The data suggest that supersensitive responses to D1 stimulation correlate temporally with striatal dopamine depletion.

539.4

DIFFERENCES OF D, AND D, DOPAMINE RECEPTOR DENSITY BETWEEN THE NEUROLEPTIC NON RESPONSIVE (NNR) AND NEUROLEPTIC RESPONSIVE (NR) LINES OF MICE - A QUANTITATIVE RECEPTOR AUTOGRAPHIC (QRA) STUDY. Y. Qian, B. Hitzemann* and R. Hitzemann. Departments of Psychiatry and Neurobiology, Stony Brook Univesity, Stony Brook, NY 11794. QRA was used to examine D, and D, receptor density in the NNR and NR lines of mice which have differed by an order of magnitude in their sensitivity (ED50) to catalepsy induced by neuroleptics with a high D/D, receptor activity profile. D, receptor density ('H-SCH23390 binding) was different in any aspect of the caudate-putamen (CPU), the nucleus accumbens (NAC) and the substantia nigra zona reticulata (SNR). D, receptor density ('H-Spiroperidol binding) was different in the central medial (CM) and the NNR line also showed a 20 to 50% elevation of D, receptor density across all the midbrain DA cell groups (A8, A9 and A10). Overall, the data show that selection of mice for response and non-response to neuroleptic-induced catalepsy is associated with changes in D, but not D, receptor density.

539.5

DENSITY OF DOPAMINE (DA) CELLS IN THE NEUROLEPTIC RESPONSIVE (NR) AND NEUROLEPTIC NON-RESPONSIVE (NNR) LINES OF MICE: RELATIONSHIP TO D, AUTO¬

RECEPTOR DENSITY. R. Hitzemann*, Y. Qian and R. Hitzemann. Departments of Psychiatry and Neurobiology, SUNY at Stony Brook, Stony Brook NY 11794 and Psychiatry Service, VA Medical Center, Northport, NY 11768.

The NR and NNR lines of mice have been selectively bred for 14 generations to yield animals with less responsivity to neuroleptic-induced catalepsy. A principal correlated response to selection appears to be an increase of D, receptor density in the NNR (see Qian et al., 1991, this meeting). This response was first noted in S1 and has persisted through subsequent generations. To test the hypothesis that the increase in D, receptor density is associated with the increase in D, receptor density, we have examined the distribution of D, receptors across the midbrain. The distribution of D, receptors in the midbrain is shown in the figure. The data suggest that the specific activity of D, receptors is markedly enhanced in the NNR line.

539.6

NEUROLEPTIC INDUCTED CATALEPSY AMONG EIGHT INBRED STRAINS OF MICE. E.J. Kanes, B. Hitzemann* and R. Hitzemann. Departments of Psychiatry and Pharmacology, SUNY at Stony Brook, Stony Brook NY 11794-8101 and Psychiatry Service, VAMC, Northport NY 11768.

Our laboratory has used selective breeding to investigate the genetic factors associated with response and non-response to neuroleptic induced catalepsy (see Qian et al., 1991). The study of multiple inbred strains provides a different strategy to understand such genetic factors. Eight inbred strains have been used in these experiments: DBA/2, CBA, C57BL, C3H, A, AKR, BALB/c and DBA/2. The ED50 values (μg/kg) among the eight strains for haloperidol-induced catalepsy were: DBA/2 4.5, C57BL 3.8, C3H 1.9, A 2.3, AKR 0.82, BALB/c 0.45, DBA/2 0.48. ED50 values for the specific D1 agonist, SCH 23390 are: LP 0.29 μg/kg; 0.15; 0.16, C57BL 0.16, C3H 0.28, A 0.70, AKR 0.36, BALB/c 0.20, DBA/2 0.32. There was no correlation between haloperidol-induced catalepsy and dopamine receptor density. These results are in progress to determine pre- and post-synaptic D, receptor density and the distribution of the dopamine receptor-expressing neurons in the regions shown to be different between the neuroleptic responsive (NR) and non-responsive (NNR) lines.
539.7

D1 AND D2 DOPAMINE (DA) RECEPTORS IN MICE WITH GENETICALLY DIFFERENT MESOTELEcephALIC DA SYSTEMS. L. L. Wagger, M. V. Catallo, C. A. Judah, Nathan Kline Institute, Orangeburg, NY 10962 and New York University Medical Center, 550 First Ave., New York, NY 10016.

The aim of this work was to test the hypothesis that in the mesotelencephalic DA system densities of striatal DA receptors are correlated with strain-dependent variations in mesotelencephalic tyrosine hydroxylase activity (TH/MES). In order to provide reliable estimates for D1 and D2 receptor properties by controlling the intra- and inter-assay variability, a homogeneous pool of reference striatal membrane was prepared, which was subjected to Scatchard analysis in each batch of assays. Kd and Bmax data for striatal samples of BALB/c, C57BL/6 and CXBl strains were corrected by factors derived from the reference. Statistical analysis indicated that each strain significantly differed in TH/MES. However, no significant strain differences were found for striatal D1 and D2 receptor binding with [3H]-Sperpine and [3H]-SCH 23390, respectively. Our results demonstrate that genetic factors determine a highly significant variation of TH/MES which is not correlated with significant genetic changes in D1 or D2 receptor densities in the striatal projection area.

539.8


Use of [3H]-U-86170F as an agonist (Ag) and an antagonist (Ant) allowed a novel approach to predicting the intrinsic activity (IA) of dopaminergic compounds.

Saturations studies using [3H]-U-86170F in rat striata and cloned D2 cells (C. Chio et al., Nature, 343:206, 1990) revealed Kd's of 1.72 ± 0.99 nM, respectively. The Bmax in D2 clones was 60-70% of that obtained with [3H]-Sperpine, and [3H]-U-86170F binding was inhibited by a haloperidol binding that it binds to the high affinity Ag state of the D2 receptor.

Evaluations of several dopaminergic agents in [3H]-Sperpine + GTP binding (low affinity Ag state) and [3H]-U-86170F binding (high affinity Ag state) were made. Ratios of the Kd values (LowAg/HiAg) correlated well with electrophysiologically derived IA values (M. Pierie et al., Society for Neuroscience, Abs 109.11, 1990). The ratio of the IA values of an agent for these receptor states provides a useful measure of IA for dopamine Ag's and partial Ag's. In addition, a correlation exists between a compound's effect on rat striatal homovanillic acid levels and its IA.

Using an Ag and an Ant ligand in cloned D2 receptor membrane preparations provides a simple and effective means of identifying dopamine Ag partial Ag, and Ant activity.

539.9


Previous data have clearly indicated that the benzazepine ring of SCH 39166, a potent D1agonist, and has induced a novel approach to predicting the intrinsic activities (IA) of dopaminergic compounds.

Saturations studies using [3H]-U-86170F in rat striata and cloned D2 cells (C. Chio et al., Nature, 343:206, 1990) revealed Kd's of 1.72 ± 0.99 nM, respectively. The Bmax in D2 clones was 60-70% of that obtained with [3H]-Sperpine, and [3H]-U-86170F binding was inhibited by a haloperidol binding that it binds to the high affinity Ag state of the D2 receptor.

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Using an Ag and an Ant ligand in cloned D2 receptor membrane preparations provides a simple and effective means of identifying dopamine Ag partial Ag, and Ant activity.
DIZOCILPINE (MK 801), BUT NOT NBOX, ALTERS Dopamine (DA)-Mediated CHANGES IN STRIATOPALLIDAL NEURONAL ACTIVITY AND BEHAVIOR. R.C. Bado, K.-X. Huang, J.F. Walters, ETB, NINDS, NIH, Bethesda, MD 20892, USA.

Altering basal ganglia glutamate (GLU) transmission may have potential for treating Parkinson’s disease. Using single-unit recording techniques, and a stereotyped rating scale, we are examining the role of GLU in regard to spontaneous neuronal activity and behavioral responses. AP0 (0.3 mg/kg, iv) significantly reduced the firing rate of 9/10 Type I caudate neurons. Following MK 801 (NM DA antagonist; 0.1 mg/kg, iv), AP0 inhibited Type I (3/10), while neuronal cells were excited on oureected, resulting in no net change in rate. Globus pallidus (GP) neuronal waveforms can be identified as Type I (+/-) or Type II (+/-. APO excited Type II GP neurons (10/10), and inhibited Type I neurons. MK 801 caused a net blockade of APO effects on Type II cells (4/10 excited, 3/10 inhibited), but had no effect on Type I neurons. MK 801 increased locomotion, but completely inhibited the stereotyped sniffing and licking induced by iv APO.

NBOX (AMPA antagonist; 1 mg/kg, iv) reduced the firing rate of 4/5 Type II GP neurons, but did not alter their responsiveness to APO.

Ketamine-anesthesia (150 mg/kg, ip), did not alter APO-blocking properties with MK 801 (only neuronal activity adjusted). Conversely, MK-801 (18.4 mg/kg, ip) did not induce anesthesia in rats.

These data suggest that normal glutamate transmission is necessary to observe DA-mediated electrophysiological effects and stereotypy. The ability of MK 801 to block APO stereotypy may be due to the observed alterations in striatal GP neuronal activity, while locomotor stimulant effects of MK 801 to block APO stereotypy may be due to the observed alterations in striatal GP neuronal activity.

DO PRESYNAPTIC Dopamine AUTO-RECEPTORS INHIBIT Dopamine RELEASE VIA ACTIVATION OF POTASSIUM CHANNELS? Takahiko Tanaka, Steven R. Vincent, George G. Nomikos and Hans C. Fibiger. Div. of Neurological Sciences, Dept. of Phys. Univ. of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., Canada, V6T 1Z3

Somatodendritic dopaminergic (DA) auto-receptors inhibit DA cell firing by activation of potassium (K+) channels via a G protein mediated mechanism. However, the signal transduction mechanisms by which presynaptic auto-receptors, and the mechanism by which the stratum inhibits DA release are not known. The purpose of this study was to examine the role of K+ channels in DA auto-receptor function in the striatum using in vivo microdialysis. DA agonists quinpirole (QPP; 10 μM) and BHT-920 (1 μM) reduced extracellular DA in the striatum. This was blocked by the DA antagonist (-)¬-sulpiride (SLP; 10 μM), which itself released extracellular DA. Lack of stimulation of K+ channel blocker quinine (QIN; 1 mM) caused a large increase in DA release which stabilized after 90 min. QIN completely blocked the SLP-induced DA release, but did not block the effect of QPP. SLP blocked the effect of QRR in both the presence and absence of QIN. These results confirm that auto-receptors in the striatum regulate DA release. The results also indicate that DA receptor agonists act via additional mechanisms that are independent of K+ channel activation to inhibit release.

D1 AGONIST HAS OPPOSITE EFFECTS ON NEURONAL TRANSMISSION IN THE BASAL GANGLIA OF TWO ANIMAL MODELS OF PARKINSON’S DISEASE: EFFECTS BLOCKED BY NM DA ANTAGONISM. K.-X. Huang and J.F. Walters. NINDS, NIH, Bethesda, MD 20892.

6-hydroxydopamine-induced lesion of the substantia nigra dopaminergic and subthalamic neurons are two strategies used for producing animal models of Parkinson’s disease (PD). We have compared the responses of substantia nigra pars reticulata (SNpr) neurons to the D1 agonist SKF-38393 in these animal models using extracellular recording techniques in locally anesthetized, gallamine paralysed rats. Effects of MK-801 were also examined since NM DA antagonists are of interest in PD. In 6-OHDA-lesioned rats (1 mg/kg/day, sc) for 6 days, SKF-38393 (10 mg/kg, iv) significantly increased the firing of SNpr neurons (ave. increase: 60 ± 15% compared with control (18 ± 8%, n=7). This effect was reversed by the D1 antagonist (-)¬-sulpiride (SLP, 10 μM), and was potentiated: SKF-38393 induced a similar change in rats treated with reserpine for 4 days then allowed a five-day washout period. As shown previously, in 6-OHDA-lesioned rats studied 9-26 weeks after lesion, SKF-38393 inhibited SNpr firing rates (ave. decrease: 75 ± 10% below baseline, n=3). Time course differences do not account for these opposite effects: seven days after 6-OHDA lesion SKF-38393 did not induce effects similar to those seen after reserpine. MK-801 (0.15 mg/kg, iv), 5 μm before SKF-38393, blocked both the stimulatory and the inhibitory effects of SKF-38393 in paralysed and lesioned rats, respectively. Results suggest that monoamine depletion and dopamine cell degeneration both affect D1 neuronal function in opposite ways, and raise questions about which model is more relevant to PD. They also support a role for tonic NM DA transmission and point to more considerable plasticity in D1 mechanisms regulating striatal output.
539.19 CHRONIC SCH 23390 ACCELERATES THE RECOVERY OF D1 DOPAMINE RECEPTOR LEVELS IN NEONATAL AND ADULT RATS FOLLOWING IRREVERSIBLE BLOKEDGE. S. G. J. G. Johnson, S. A. Crawford, S. A. McDougall* & M.T. Bardo. Department of Psychology & Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506.

The present study was designed to investigate the effect of a non-competitive D1 receptor antagonist, SCH 23390 (0.05 mg/kg, s.c., three times a day for three weeks). The process of receptor recovery, from the irreversible inactivation of D1 receptors, was examined. SCH 23390 significantly enhanced the steady state density of D1 receptors in the striatum (+24%). Chronic treatment with SCH 23390 also increased the production rates of D1 receptors in the ST and in the SN (+24%). These results are consistent with the view that an increase in the receptor production rate may be due to an increased expression of the genes that control the production of D1 DA receptors.

539.20 DEVELOPMENTAL DIFFERENCES IN D1 AND D2 DOPAMINE RECEPTOR DESTRUCTION AND DEPOLARIZATION IN RATS AFTER IRREVERSIBLE ANTAGONIST TREATMENT. C. J. McDougall*, N. Elkins* & M.T. Bardo. Department of Psychology & Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506.

Previously, our lab reported developmental differences in the behavioral responses to treatment with the irreversible dopamine D1 receptor antagonist SCH 23390 (75 mg/kg, s.c., three times a day for three weeks). The process of receptor recovery, from the irreversible D1 receptor inactivation, was examined. SCH 23390 significantly enhanced the steady state density of D1 receptors in the striatum (+24%). Chronic treatment with SCH 23390 also increased the production rates of D1 receptors. The authors concluded that an increase in the receptor production rate may be due to an increased expression of the genes that control the production of D1 DA receptors.

539.21 STRIATAL DOPAMINE RECEPTOR AND DOPAMINE LEVEL DEPRESSION IN NEONATAL AND ADULT RATS FOLLOWING IRREVERSIBLE RECEPTOR ALKYLATION. J.E. Rockwell, C.A. Crawford, S.A. McDougall* & M.T. Bardo. Department of Psychology, University of Kentucky, Lexington, KY 40506.

The present study assessed the effects of a non-competitive dopamine receptor antagonist, SCH 23390 (75 mg/kg, s.c., three times a day for three weeks), on D1 and D2 dopamine receptor number (Bmax) and dopamine (DA) levels in 16 day old and adult rat striatum. Dopamine receptor binding was assessed in homogenates prepared at 24, 48, 96 or 192 hrs after EEDQ treatment. Rats received a single injection of EEDQ (7.5 mg/kg) or vehicle when 10, 16 or 39 days old. Results showed that EEDQ produced an initial depletion of D1 and D2 receptors in all age groups. This effect tended to be less profound in both 10- and 16-day old rats when compared to the 39-day-old rats. For all ages, evidence for receptor recovery was obtained.


Alterations in dopaminergic (DA) systems have been suggested as one neurochemical mechanism for Pb-induced behavioral effects. Recent work has shown DA receptor number (Bmax) to be elevated in the striatum (ST), but decreased in the nucleus accumbens (NA) after postnatal Pb exposure. The present study was designed to investigate the effect of Pb exposure on the development of DA D1 and D2 receptors. The authors concluded that Pb exposure produces differential developmental effects on DA receptors. For postnatal (PN) exposure, lactating dams received 0, 100, 350, 1000 or 2000 ppm Pb lead acetate in drinking water; postweaning (PW) Pb exposure was 0, 50 or 250 ppm Pb acetate. Rats were sacrificed on 7,14,21 or 60 days of age for PN exposure, and on days 28 or 60 for PW exposure. The authors concluded that Pb exposure produces U-shaped dose-response functions for both D1 and D2 receptors, with ST curves somewhat left-shifted relative to NA, suggesting a more pronounced effect of Pb on striatum. At neither day 21 or 60 was there evidence of differential Pb accumulation in ST vs NA, suggesting that in PN-exposed rats, differential Pb levels was not the mechanism of the differential sensitivity. By 60 days of PN-exposed rats, U-shaped rather than inverse U-shaped Bmax curves were apparent, indicating a significant increase in D1 receptors at the highest exposure level. Pb was still present in a dose-related manner at day 60 in both ST and NA, suggesting Pb might still be actively affecting the tissue. This work was supported by NIH grant ES08017.

CATECHOLAMINES: Dopamine II

540.1 Dopamine actions on Nebrebrad conductances in rat ventral tegmental area (VTA) neurones. Z.G. Jiang and R.A. North. Volium Institute, Oregon Health Sciences University, Portland, OR 97201.

Whole cell recordings were obtained from 120 VTA neurones in rat midbrain slices. Pipettes contained potassium as the main cation, and ATP and CTP. First, dopamine (DA 50 nM, EC50 = 22 μM) current, an outward current in 56% of the cells; this was typically about 50 pA. The current reversed to inward at -105 mV, close to predicted reversal for DA current. There was no association with an increase in instantaneous conductance at +60 mV to 2 nS (27% of control, n = 14). Second, a voltage-dependent inward current that was sensitive to TEA (10 mM) and barium (100 μM) was increased by dopamine (by 18% at 0 mV). Third, dopamine reduced a hyperpolarization-activated current at -120 mV, with no obvious shift of its voltage-dependence. Dopamine had no effect on a transient outward current (TA). Thus, dopamine inhibits VTA principal neurones both by increasing two potassium conductances and reducing h.


Dopamine neurones were identified by preloading with 5,7-DHT (15-50 μM for 1 hr) and visualization under UV fluorescence. This resulted in a blue-violet fluorescence within the soma and dendrites. To study only calcium (Ca2+)-dependent inward current, the extracellular solution contained 140 mM tetraethylammonium Cl, 10 mM CaCl2, 1 mM MgCl2, and 10 mM glucose (pH 7.4). The patch pipette solution contained 10 mM EGTA, 135 mM CaCl2, 1 mM MgCI2, 1 mM GTP, 2 mM ATP, 14 mM creatine phosphate, 50 μM creatine kinase. Both solutions were buffered to pH 7.4 with 10 mM HEPES. Depolarizing voltage steps from a holding potential of -90 mV resulted in inward currents which were observed at membrane potentials between 40 and 20 mV. These currents were not observed when Ca2+ was removed from the external solution. The whole-cell current displayed an initial peak outward component followed by a biexponential time course with a characteristic inward current of lower amplitude which lasted for the duration of the voltage step (500 msec). This current response suggests the presence of at least two Ca2+ currents in cultured DA neurones, one which is transient and a second which is sustained. Studies designed to isolate and characterize these inward currents are in progress. It was noted that the presence of inward currents was correlated with the age of the cultures. Cultures were studied between 8 and 19 days, however, inward Ca2+ currents were only present in culture which were at least 13 days old. (Supported by MH41557 and NS26291.)
540.3
EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON SUBSTANTIA NIGRA DOPAMINE NEURONS
Fetal Alcohol Syndrome is a disorder which is associated with altered spontaneous motor activity and increased motor impulsivity. Some of the effects detailed in the literature are reminiscent of behaviors thought to be mediated, in part, by dopaminergic (DA) neuronal systems within the brain. In an attempt to better understand the neurobiological underpinnings of this syndrome, we have begun to study the effects of in utero exposure of rats to ethanol on the subsequent physiology and pharmacology of mesencephalic DA neurons in vitro. Pregnant rats received a liquid diet containing 0%, 17.5%, and 35% ethanol observed calories from day 8 of pregnancy until delivery. Total caloric intake was the same for all groups. At birth, pups were transferred to foster dams.

Single-unit extracellular recordings were made from identified DA neurons located within the substantia nigra, two to three months after weaning. DA neurons in rats treated with 17.5% and 35% ethanol exhibited slightly higher spontaneous firing rates and burst-related action potentials compared to controls. DA neurons in these rats also were dramatically less sensitive to the inhibitory effect of intravenous administration of the mixed D1/D2 receptor agonist, quinpirole. This was observed as both a significant increase in the Δτp (144 μg/kg for the combined 17.5% and 35% ethanol groups vs. 16 μg/kg in controls) as well as a dramatic reduction in efficacy, suggesting a functional down-regulation of DA receptors. Thus, prenatal exposure of rats to ethanol affects the physiology and pharmacology of nigral DA neurons ultimately observed in the adult animals. (MH41557 and PS0 A07066).

540.5
RELEASE OF CHOLECYSTOKININ FROM RAT MIDBRAIN SLICES
Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48201.
Cholecystokinin octapeptide (CCK-8) is present within a majority of rat midbrain dopamine (DA)-containing neurons. Exogenous CCK-8 can modulate DA neuronal electrophysiology, in part, by direct actions on the somatodendritic region of these cells. Thus, if CCK-8 is released somatodendritically from DA neurons, it may influence DA cell function as well. In the present study, a 5-HT (serotonin, 1 μM/liter) bath was used to measure the in vitro release of CCK-8 from midbrain slices (400 μM). The slices obtained from a 2 mm coronal slab of trimmed rat midbrain were used in each assay tube. All CCK-immunoreactivity was established by HPLC to be authentic CCK-8.

Low levels of CCK-8 were detected in the basal incubation medium. Thirty mM potassium caused about a 3-fold increase in the release of CCK-8. This stimulated release was abolished in calcium-free medium. The D2 DA receptor agonist quinpirole (1-100 μM), but not the D1 agonist SKF 38393 (10-100 μM), attenuated the potassium-stimulated release of CCK-8 without altering basal release. The quinpirole effect was prevented by the D2 antagonist I-SU14803 (10 μM) and was not antagonized with SKF 38333 (10 μM). These results show that CCK-8, like DA, can be released from midbrain slices, presumably from DA/CCK-8-containing neurons. This finding is in accordance with the possibility that CCK-8 plays a role in the regulation of DA neuronal function at the level of the cell body, where it might influence DA cell excitability. (Supported by MH41557, MH42136 and MH43026).

540.7
EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OH-DPAT) ON THE ELECTRICAL ACTIVITY OF DOPAMINE NEURONS IN THE RAT VENTRAL TEGMENTAL AREA
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Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Gest, S. Maria Imbaro (CH), Italy.
A number of studies have shown that serotonin (5-HT) containing neurons in the raphe nuclei exert an inhibitory influence upon dopaminergic (DA) neurons in the substantia nigra, pars compacta (SNc). The selective 5-HT1A receptor agonist 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT) (8-OH-DPAT) has been shown to increase the firing rate of DA neurons in the SNc, probably as a consequence of its inhibitory action on 5-HT containing neurons in the dorsal raphe nucleus. Neuronal autoradiographical data indicate that DA neurons in the ventral tegmental area (VTA) receive an excitatory GABAergic input from the raphe nuclei, however the functional role of serotonin in the control of the activity of mesoencephalic DA system is still unclear.

In the present study, extracellular single unit recordings were performed in male Sprague Dawley rats, anesthetized with chloral hydrate. Dopamine neurons in the VTA were recorded using single barrel micropipettes and were identified by their location, waveform, firing rate and pattern. Intravenous injection of 8-OH-DPAT (10-160 μg/kg; cumulative dose) caused excitation in about 40% of the neurons studied; in 20% of the cells recorded a slight inhibition was observed; the remaining cells were unaffected by 8-OH-DPAT. These findings indicate that the activity of a subpopulation of DA neurons in the VTA is under the control of the serotonergic system. Experiments are under way to establish whether the effect of 8-OH-DPAT on VTA DA neurons is mediated by an inhibitory action on 5-HT-containing neurons in the nucleus raphe dorsalis (DR) and/or medianus (MR).

540.4
EFFECTS OF SIGMA LIGANDS ON THE POPULATION ACTIVITY OF MIDBRAIN DA NEURONS.
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Sigma receptors have been hypothesized to play a role in the etiology of psychosis and in the therapeutic effectiveness of antipsychotic drugs. Several sigma ligands also produce psychotomimetic effects although the receptor specificity of these responses is not clear. In order to determine if the DA and A10 dopaminergic (DA) systems are believed to mediate the side effects and therapeutic effects, respectively, of antipsychotic drugs. In the present study, we evaluated the effects of three sigma ligands, (+)-pentazocine (PZ), DTG and JO 1784, on the numbers of spontaneously active A9 and A10 DA neurons encountered per electrode track in anesthetized rats. Acute injections were given 1 hr prior to electrophysiological recordings. Chronic treatment consisted of 21 daily injections with a 20 hr withdrawal. Each treatment group contained 10 rats. Acute i.p. PZ (10 mg/kg), DTG (2 mg/kg) and s.c. JO 1784 (1 and 10 mg/kg) did not alter the numbers of spontaneously active DA cells per electrode track. Chronic treatment with PZ (1 and 10 mg/kg) or DTG (0.2 and 2 mg/kg) increased the numbers of A10 DA cells per track. At 1 mg/kg, chronic JO 1784 had no effect on the number of DA cells per track. At 10 mg/kg, chronic JO 1784 moderately decreased the number of active A9 DA per track and increased the firing rate of A10 DA cells. Thus, repeated but not acute treatment with these sigma ligands altered the population profile of active DA neurons. These effects differ from those of typical and atypical antipsychotic drugs which inactivate A10 DA neurons upon chronic administration. (Supported by MH41536).

540.6
DOPAMINE NEURON ONTOGENY: MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS
D.K. Pitts 1,2, J. Rybin 1, A.S. Freeman 2 and L.A. Chiodo 2.
Antidromically (AD) identified rat nigrostriatal dopamine (DA) neurons undergo significant physiological and pharmacological changes during postnatal (PN) development (Pitts et al. Synapse 8:309, 1990; Tepper et al. Dev. Brain Res. 54:21, 1990). The distribution of tyrosine hydroxylase-immunopositive (TH+) neurons and the electrophysiological characteristics of AD identified mesoaccumbens DA (MADA) neurons during PN development were examined in the present study. As previously described (Tepper et al. Neuropsych Abstr. 43:111, 1990), on PN day (PND) 14 TH+ neurons and fibers were found scattered throughout the substantia nigra with a subsequent reduction in TH+ somata/fibers ventrally by PND 14 resulting in the appearance of a more adult-like non-DAergic pars reticulata. In addition, at PND 1 more posterior-lateral and anterior-medial groups of TH+ neurons were observed which may ultimately contribute to the adult mesencephalic DA neuron population. Preliminary studies of AD identified MADA neurons at PND 14-16 suggest that there are fewer cells with a bursting discharge pattern and that the burst duration is shorter relative to adults. (Supported by MH75877 [DKP], MH42136 [ASF], MH15577 [LAC]).

540.8
GAMMA-HYDROXYBUTYRATE INCREASES THE FIRING RATE OF A9 DOPAMINE NEURONS IN UNANESTHETIZED RATS.
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Gamma-hydroxybutyrate (GHB) is a normal metabolite of the mammalian brain and is found in discrete cerebral regions such as the substantia nigra (SN). However GHB receptors are virtually absent from the striatum and hippocampus. For these and other reasons GHB is a molecule of great interest especially in light of recently reported evidence which suggests in GHB a role in the treatment of alcohol abusers (Hallowitz et al. 1990 Lancet). Since electrophysiological effect of the drug has always been explored in anesthetized rats and anesthesia alters many drug-induced effects we studied the effect of GHB in unanesthetized rats. Male Sprague-Dawley (200-250 g) rats were temporarily anesthetixed with a mixture of ether inhaled with a head held with a head holder for artificial respiration and in the femoral vein for intravenous administration of drugs. Gamma-hydroxybutyrate (400 μg/kg iv) was administered and once muscular paralysis was obtained the rats were placed on a stereotaxic frame (Bip). Low doses of GHB (50-600 μg/kg) dose dependently increased the firing rate of A9 dopaminergic neurons from about 10 to about 50% above baseline. When GHB was administered intraperitoneally higher doses (750 μg/kg) only a mild reduction of firing rate was observed. In contrast, in chloral hydrate anesthetized rats, 200 μg/kg of iv GHB failed to modify neuron activity markedly while it increased neuron activity markedly in unanesthetized rats. The results indicate: 1) anesthesia alters the electrophysiological effects of GHB; 2) GHB affects the effects of ethanol upon dopaminergic neurons in both unanesthetized and anesthetized rats.

Systematic administration of the dopamine (DA) antagonist haloperidol (HAL) increases the firing rate of nigral DA neurons. This excitation has been suggested to result from the blockade of DA receptors located on striatal neurons projecting to the substantia nigra. HAL also may exert local effects within the nigra by blocking somadendritic release of DA, which is localized in the DA neuron terminals. To determine whether this effect is due to HAL, we measured the increase in the tonic firing rate of DA neurons in the SNC and VTA in response to local perfusion of HAL in the substantia nigra. In all studies, HAL was applied locally to a small number of DA neurons in the SNC and VTA. In this study, we compare the effects of these two compounds on DA release and metabolism in the nucleus accumbens and in the caudate nucleus with haloperidol and haloperidol, in the microdialysis model. We show that (+)-A76 is more effective than SCH 23390 in the microdialysis model. We show that (+)-A76 is more effective in the ICSS model than SCH 23390 but has no effect on the response curve to the right, i.e., acts inhibitory. The subthreshold method is used to determine the presence of a hyperpolarizing effect of (+)-A76. The results of this study confirm the hypothesis that a hyperpolarizing effect of (+)-A76 is due to the blockage of DA autoreceptors located on the terminals of the striatonigral projection. In conclusion, the results of this study suggest that (+)-A76 blocks the stimulatory effects of DA on DA autoreceptors and that these effects contribute to the increased firing rate of nigral DA neurons.

REFERENCES


540.11 BLOCKADE OF SOMADENDRITIC AUTORECEPTORS ON NIGRAL DOPAMINE NEURONS CONTRIBUTES TO THE FIRING RATE-INCReasing EFFECTS OF DOPAMINE ANTAGONISTS. M.L. Pucak and A.A. Grace. Deps. of Behavioral Neuroscience and Psychiatry, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Systemic administration of the dopamine (DA) antagonist haloperidol (HAL) increases the firing rate of nigral DA neurons. This excitation has been suggested to result from the blockade of DA receptors located on striatal neurons projecting to the substantia nigra. HAL also may exert local effects within the nigra by blocking somadendritic release of DA, which is localized in the DA neuron terminals. To determine whether this effect is due to HAL, we measured the increase in the tonic firing rate of DA neurons in the SNC and VTA in response to local perfusion of HAL in the substantia nigra. In all studies, HAL was applied locally to a small number of DA neurons in the SNC and VTA. In this study, we compare the effects of these two compounds on DA release and metabolism in the nucleus accumbens and in the caudate nucleus with haloperidol and haloperidol, in the microdialysis model. We show that (+)-A76 is more effective in the ICSS model than SCH 23390 but has no effect on the response curve to the right, i.e., acts inhibitory. The subthreshold method is used to determine the presence of a hyperpolarizing effect of (+)-A76. The results of this study confirm the hypothesis that a hyperpolarizing effect of (+)-A76 is due to the blockage of DA autoreceptors located on the terminals of the striatonigral projection. In conclusion, the results of this study suggest that (+)-A76 blocks the stimulatory effects of DA on DA autoreceptors and that these effects contribute to the increased firing rate of nigral DA neurons.

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540.13 THE Dopamine (D3) Preferring Autoreceptor Antagonist (+)-A76 and (+)-UH232 Produce Conditioned Place Preference in the Rat. J. Ekelin, S. Tlemeany and C. Trangholm. Dept. of Pharmacology, Univ. of Göteborg, PO Box 33031, S-400 33 Göteborg, Sweden

The preferential dopamine (D3) autoreceptor antagonists (+)-A76 and (+)-UH232 increase the turnover and number of spontaneously active dopaminergic neurons in the SNC and VTA. This effect is observed even in the absence of a locomotor response. These results are consistent with the hypothesis that a hyperpolarizing effect of (+)-A76 may be due to the blockage of DA autoreceptors located on the terminals of the striatonigral projection.

REFERENCES


Dopamine (DA) synthesis can be regulated in situ by stimulation of nerve terminal DA autoreceptors (AR). Using striatal brain slices, we have examined the ability of different classes of DA antagonists to affect the inhibition of DA synthesis induced by DA agonists in vivo. Stimulation of DA AR's was measured as the inhibition of K+ stimulated DOPA accumulation by a fixed dose of either quinpirole or 7-OHDPAT. The rank order for reversal of 1 μM QUIN was PD-128483 (1 μM) > (-)-UH232 = (-)-AJ76 > (-)-UH242. Although (+)-AJ76 was equipotent with CLOZ against QUIN. (+)-AJ76 was more efficacious than CLOZ when tested against 7-OHDPAT (1 μM). PD 128483 (10 μM) partially reversed the effect of QUIN under these conditions. Under basal conditions (+)-UH232 was equipotent with PD-128483 and (-)-3PPP, but not (+)-AJ76. (+)-UH2232, or (+)-UH242, exhibited AR agonist properties. Under depolarizing conditions (K+ = 30 mM), neither PD 128483 nor (-)-3PPP inhibited DOPA accumulation. Compounds do not interact with the DA transporter since neither PD 128483 nor the antimeotransin (3 μM) blocked the amphetamine (3 μM) induced release of DA. The effects of PD 128483 at DA autoreceptors appear to mimic those of the partial agonist (-)-3PPP. Furthermore, our results confirm that (+)-AJ76 and (+)-UH232, putative D3 receptor antagonists, are antagonists at synthesis modulating DA AR's. Supported by DA-04120 and Michigan Department of Mental Health.

EVIDENCE FOR DOPAMINE D2 AUTORECEPTORS IN NEONATAL AND INFANT RAT PUPS. C.A. Moody, H. Radice*, L.P. Sear, Dept. of Psychology and Center for Developmental Psychobiology, SUNY, Binghamton, NY 13902-6000.

The presence of functional dopamine (DA) D2 autoreceptors was assessed in neonatal (postnatal day 3- P3) and infant (P10) rat pups. 35 min prior to sacrifice pups received a 750mg/kg s.c. injection of gamma-butyrolactone (GBL) to block impulse flow in DA neurons. 30 min prior to sacrifice all pups received a 100mg/kg i.p. injection of the dopa decarboxylase inhibitor 3-hydroxyphenylpyrindazone, with this increase in the quinpirole group receiving a s.c. injection of 0.5mg/kg quinpirole immediately thereafter. Following decapitation, forebrain and striatal regions were dissected on ice from P3 and P10 brains, respectively, and frozen for later assessment of DOPA concentrations via HPLC/sec. At both ages GBL treatment increased DOPA levels, with this increase being blocked by quinpirole. This agonist induced production of DOPA levels provides evidence that D2 presynaptic autoreceptors are functional in terms of inhibiting DA synthesis in young animals. In addition, preliminary findings suggest that the D1 agonist SKF-38393 may also inhibit DA synthesis early in life. Further investigation of dopamine D2 autoreceptors in the neonatal rat may provide insight into the physiological pharmacology of the physiologic Leech-Nyhan disease. Supported by NIMH 082717 and NICHD 08001.
540.21

BHT 920 SELECTIVELY STIMULATES A SUBTYPE OF D-2 RECEPTORS COUPLED WITH VOLTAGE-DEPENDENT K' CHANNELS. A. Valarini, M. Pizzii, M. D. Carruba*, G. Esposito, N. Banarese*, M. Meso, P.F. Spano, Dep. Biom. Sci. & Biotech., Section Pharmacol. Exp. Ther. School of Medicine, Genoa, Italy. Radioactive rubidium efflux was used to measure potassium conductance. The study designed to assess both the presence and the sensitivity to ions and drugs of the K' channels located in the pre synaptic nerve terminals of the striatum. Our data provide evidence for the existence of at least two different K' channels which participate differently in the regulation of K' fluxes under both resting and stimulated conditions. The two different components of K' fluxes were found on the basis of their kinetic properties and sensitivity to calcium ions. We also found that both the K' channels present in the isolated nerve terminals are sensitive to dopamine and various, but not all, D-2 dopaminergic drugs. In particular, the aspine derivative BHT 920 discriminates between the two channels behaving as selective opener of the voltage-dependent, calcium-insensitive K' channels. Our results support the conclusion that presynaptic nerve terminals of rat striatum possess two distinct categories of K' channels: a voltage-dependent and a calcium-activated K' channel. The two different K' channels serve as effectors of two distinct subtypes of dopamine D-3 receptors.

540.22

NEUROCHEMICAL EFFECTS AND SELECTIVE DISTRIBUTION OF AMISULPRIDE IN THE MESOLIMBIC DOPAMINERGIC SYSTEM AFTER CHRONIC TREATMENT WITH D-2 RECEPTOR ANTAGONISTS. M. Schorderet, S. Touvron, J. Margetit* and C. Jacquot, Fac Pharmacie, SDS 6313, 29296 Chateany-Malabry, Lab Delagrange, 91380 Chilly-Mazarin, France. We have previously shown that low-dose (2mg/kg, i.p.) amisulpride (A), an atypical neuroleptic and D2 receptor antagonist, significantly reduced the concentration of 3MT (an index of dopamine release) in the nucleus accummbens (NAc) without modifying DOPAC or HVA concentrations. No effect of the drug was observed in the striatum. Our data are in agreement with the results of others, e.g., 1988, Ann. Psychiatr, 3: 284-297. In this study, we determined whether the AMT increase persisted after 14 days of treatment and whether the localized effect of A in the NAc was associated with a preferential distribution of the neuroleptic in this structure. Male rats treated with A or treated with D-1 agonist SKF 38393 (S) and the D2 agonist quinpirole (Q), or the dopamine releaser d-amphetamine (A), into the NAc. Two groups of male Sprague-Dawley rats were implanted with guide cannulae (ML: ±1.5; DV: -5.8) aimed at different sections of the NAc (AP: +2.5; ML: ±1.5). Following placement of the locomotor stimulating effects of coadministration of various doses of S or Q into the nucleus accumbens (NAc) without modifying DOPAC or HVA concentrations. In contrast, the rats showed a high locomotor response (>30 c/h) to S/Q, whereas only 3 rats showed a moderate response (100-500 c/h) and one rat failed to respond to the S/Q combination (100 c/h). In contrast, twenty rats with ANT placements displayed inconsistent locomotor effects to S/Q combinations (Mean = 307 ± 130.0 c/h). Ten rats failed to show increases in locomotor activity; two rats showed moderate increases in locomotion; and eight rats displayed high locomotor activity. The pattern of enhanced locomotor response in animals with central versus anterior accumbens placement was also evident in the response to A (200-500 c/h; ANF: 387.2 ± 45.6 c/h). These data suggest that the receptors mediating the locomotor responses to dopamine agonists in the NAc are located in the central to posterior portion of the nucleus. Supported by USHIS grant GM 34781.

540.23

DOPAMINE LEVELS IN CULTURED RAT MESENCEPHALIC NEURONS. S. Schinelli, M. Paolillo*, M. Quattieri* and G. L. Corona*, Institute of Pharmacology, University of Pavia, 27100 Pavia, ITALY. Dopaminergic neuronal account for a few per cent of the cells dissociated from embryonic midbrain and therefore the measurement of endogenous dopamine (DA) and its metabolites requires a sensitive assay. We have developed an HPLC method coupled to coulometric detection to measure the levels of DOPAC and DA in rat mesencephalic cultures. Papain dissociated mesencephalic cells from 14-15 days old rat embryos were seeded in 24 multiwell plates at a density of 300,000 cells/well. The cultures were grown in 15% horse serum and then incubated up to 2 weeks in vitro. Cellular content of DA and DOPAC was monitored at 3,6,9 and 12 days. Dopamine levels increased linearly with time of incubation ranging respectively from 2.5 pmol/well and 0.12 pmol/well at 3 DIV to 38.1 pmol/well and 2.3 pmol/well at 12 DIV. The DA/DOPAC ratio remained constant during the time of development in vitro. The levels of DA were significantly increased when mesencephalic neurons were cocultured with target striatal cells and furthermore cellular DA content in cocultures was directly correlated to the number of target striatal cells.

540.24

ANATOMICAL HETEROGENEITY OF LOCOMOTOR RESPONSES TO D1/D2 AGONISTS AND AMPHETAMINE IN THE NUCLEUS ACCUMBENS. W. K. Haman, F. Morinigoni and J. L. Lacki, Dep of Psychology, University of Pennsylvania, Philadelphia, PA, 19104. The nucleus accumbens (NAc) plays an important role in the locomotor effects of dopaminergic agents. The present study provides evidence for an anterior/posterior regional heterogeneity for induction of locomotion by coinjections of the D1 agonist SKF 38393 (S) and the D2 agonist quinpirole (Q), or the dopamine releaser d-amphetamine (A). Two groups of male Sprague-Dawley rats were sacrificed 60 min after the last injection. DA, DOPAC, HVA and 3MT concentrations were measured in the NAc and the ST, following chloroform extraction, by HPLC coupled with electrochemical detection. Trough Ami concentration was determined simultaneously in the NAc and the ST, showing ratio of locomotor activity localized to the NAc was associated with a higher concentration of Ami in the NAc than in the ST (21.3±3/g vs. 3.4±1.8/g respectively). These results support a selective involvement of the NAc in the effect of low-dose Ami and suggest that Ami may have a high affinity for a receptor subtype located preferentially in the NAc and involved in the release of DA.

540.25

A COMBINED GOLGI-STAINING AND IMMUNOCYTOCHEMICAL STUDY OF NEURONS CONTAINING DARPP-32, A DOPAMINE-REGULATED PHOSPHOPROTEIN, IN ANTERIOR CINGULATE CORTEX. C. L. DiStefano. Psychology Department, Florida State University, Tallahassee, FL 32306. Many neurons in layer VI of the rat cerebral cortex contain DARPP-32, a dopamine and cyclic AMP-regulated phosphoprotein enriched in cells containing the D1 dopamine receptor. The present study was undertaken to determine whether DARPP-32-containing neurons in anterior cingulate cortex (ACC) represent a single morphological type. Male Sprague-Dawley rats (150-250 g) were anesthetized with sodium pentobarbital (60 mg/kg) and perfused with fixative. Brain sections were immunolabeled for DARPP-32 and then Golgi-stained. After the neurons were prelude, the over stain was removed with sodium thiosulfate. At least 5 morphological cell types, including modified pyramids, an aspiny neuron, an inverted pyramid, a bipolar cell and a horizontally oriented cell were then classified as immunocytochemically distinct. These results indicate that DARPP-32 is present in many cell types in layer VI and suggests that dopamine and cAMP module layer VI cells that have different functions.

540.26

BIOCHEMICAL AND PHARMACOLOGICAL MODULATION OF THE DOPAMINE SENSITIVE ADENYLATE CYCLASE IN HOMOGENATES OF BOVINE RETINA. M. Schorderet, S. De Raad* and S. Ofori, Dep. of Pharmacology, CMU, 1211 Geneva 4 and School of Pharmacy, B.E.P., CH 1005 Dorigny-Lausanne. The effects of various biochemical factors or pharmacological agents on the dopamine (DA)-induced accumulation of cAMP mediated by the stimulation of DA D-1 receptors were investigated. The present study of Markstein et al. (J. Neurochem. 69, 1978, 1979) was modified as follows in order to optimize the response to DA: 1) 100 μM of retinal homogenate were added to 350 μM of the buffer (48.8 μM Tris-HCl, 2 mM MgCl2, 0.45 mM EGTA, 0.1 mM IBMX, pH 7.4) containing dopamine agonists or -antagonists or forskolin, except in the controls. The mixture was kept at ice-temperature during 13.5 min before a rapid conditioning of 1 min at 30°C. The final 3 min incubation was then added with 50 μM of 5 mM ATP/1 μM GppNp. After centrifugation (3300g) the pellets and supernatants, respectively and the supernatants, respectively. Under these optimal conditions, reproducible dose-response curves for cAMP formation to DA for forskolin were obtained. In addition, the system was successfully tested to study the DA D-1 agonist properties of a new ergotide (CTY 208-245) and to reassign the D-2 agonist properties of a new ergotide (CTY 205-245). These results would confirm the usefulness of bovine retinal homogenates for 1) the study of DA-sensitive adenylyl cyclase; 2) the screening of DA D-1 agonists or antagonists.
540.27

A DOPAMINE MICROCULTURE SYSTEM FOR IN VITRO DRUG TOXICITY TESTING
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Primary neuronal cultures have become an increasingly popular experimental method in examining the inhibitory or stimulatory effects of neuroactive agents. We have already established the viability and functional properties of primary mesencephalic dopamine (DA) microculture system for drug toxicity studies in our laboratory. In this study, we establish a similar culture system with dopamine (DA) neurons to compare the effects of various drugs on two neurochemical systems. A mesencephalic tangential slice containing the majority of DA neurons was dissected from gestational day 14 fetal rat brains (age established by crown-rump measurement) in Minimal Essential Medium (MEM) with antibiotics. Slices were mechanically dissociated and plated onto poly-L-lysine coated 6-well microculture plates (NUNC). The optimal initial plating density was found to be 0.8-1.0 x 10^6 cells/cm^2. Cell viability and fiber outgrowth were assessed using [3H]-DA (50 nM) uptake after 5 days culture.

Nocturnal accumulation of DA was determined by the addition of 5x10^-6 M nonselective, a specific dopamine uptake blocker.

We assessed the specificity of our culture system by examining the effects of both DA and 5-HT specific drugs on DA fiber outgrowth inhibition or enhancement. Drugs were added on day (D) 2 of culture and [3H]-DA uptake done on D5. Cocaine, MDMA, nomifensine and fenfluramine, a serotonergic releaser, did not inhibit uptake development unless added concentrations exceeded 10^-6 M. Interestingly, cocaine is not as potent as nomifensine in inhibiting DA fiber outgrowth, indicating that the release properties of cocaine distinguish it from the reuptake blocker. We are currently investigating other drugs' effects on cultured dopamine neurons. Supported by NIDA contract # 271-A7-A144.

541.1

IDENTIFICATION OF A NETWORK OF NEURONS IN THE ABDOMINAL GANGLION OF Aplysia THAT ARE IMMUNOREACTIVE FOR THE R15α  PEPTIDES. J. Koester

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The endogenously bursting, peptidergic neuron R15 expresses the R15α  gene, which encodes 3 neuropeptides: R15α 1, R15α 2, and R15β. The mRNA precursor transcribed from the R15 gene is alternatively spliced in other neurons, resulting in an mRNa that encodes a partially overlapping set of peptides: R15α 2, R15α 3, and R15α 4 (Back et al., 1987). The R15α 2 peptide has been shown to cause water retention (Weiss et al., 1989) and both the R15α 2 and R15α 3 peptides have cardioexcitatory effects. We have previously found that in the abdominal ganglion only 5 cells are strongly immunoreactive (IR) for the R15α 1 and R15α 2 peptides. Two have previously been identified: R15 (which contains the R15α 1 peptide) and the cardioexcitatory R15α 2 peptide. The purpose of this study was to identify the other 3 IR neurons, all of which stain selectively for the R15α 2 peptide.

The 3 remaining R15α 2-IR neurons have been identified. They are named L40, L4a, and L4b. The 2.9 μm cells project out the siphon nerve, and preliminary evidence suggests that they are the L9 gill motorneurons. The L40 cell is a newly characterized neurosecretory neuron that projects to the head ganglia. It excites other R15α 2 cells by strong exciting interneuron (XII), which directly excites R15, R15β, and the 2 L9 cells. Moreover, L40 is excited by L10, which also excites the other 4 cells that are IR for the R15α 2 peptide. Thus, all of the 5 cells in the abdominal ganglion that are strongly IR for the R15α 2 peptide appear to belong to the same functional network.

Previous work has implicated R15, L10 and R15β in the control of water balance and kidney function. We are attempting to test this hypothesis and to determine the roles of the L40 and L9 cells in this context.

541.3

PEDAL PEPTIDE MODULATES A SODIUM CURRENT IN NEURONS L2-L6 OF Aplysia. W. L. Pearson & P. E. Lloyd

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Application of pedal peptide (Pep) has been shown to depolarize the left upper quadrant (LUQ) neurons (L2-L6) of Aplysia. Several Pep-induced ionic currents contribute to this depolarization. Using voltage clamp and ion substitution techniques, we have isolated and identified one of the Pep-induced currents as a Na^+ current (INa(Pep)).

Pep (2.5 μM-15 μM) was bath applied to axotomized LUQ cells bathed in "Ca++-free" saline. Voltage-clamp experiments showed that Pep induced a large inward current that reversed near 20 mV. At negative potentials the current displayed pronounced rectification, so that the 1-V below -30 mV. Subtracting strychnomycin for Na^+ in the saline reduced the current and shifted the reversal potential to lower potentials, indicating that the current is cadmium resistant by Na^+ and is blocked by Na^+. In the presence of 5x10^-6 M pep., 0.2-10 μM Ca^2+ and Co^2+ produced a similar reduction of INa(Pep) at physiological Ca^2+ concentrations. INa(Pep) is largely blocked by 1 μM pep. at ionic currents.

The biophysical properties of INa(Pep) are very similar to the properties of cyclic nucleotide-gated currents characterized in a wide variety of cells, including other Aplysia neurons. Isochronic injection of cyclic nucleotides evoked a current resembling pep. treating LUQ cells with IBMX, a phosphodiesterase inhibitor, enhanced INa(Pep), indicating that a cyclic nucleotide may be involved in modulating the activity of this current. Supported by NS 23569.

541.4


Imtrinsic buccal muscle 5 (5), also the ARC is innervated by 2 cholinergic neurons which have axons in buccal nerve 3 (N3). The metabolism of 3H-choline (3H-ACh) in 5 was studied. 5 contains a high affinity uptake system for 3H-ACh which is then found entirely as 3ACh (presumably in cholinergic synapses) and is active into the nM range. N3 stimulation increases 3Ch uptake, metabolism of 3Ch is active into the nM range. N3 stimulation increases 3Ch uptake, but there is no effect on hydrolysis of 3Ch in the absence of any AChE inhibitor. Second, when N3 is stimulated with cold choline (≤100μM) indicating that little hydrolysis of 3Ch occurs. Finally, N3 stimulation depletes only 3AcH. The working hypothesis is that L2 activity modulates the metabolism characteristics of the heart wall. Because L2 is inhibited by L10, we hypothesize that its actions in the heart oppose the actions of L10 that are mediated by R15β.

Current work is directed towards identifying more precisely the sources of the serotonin-IR and the R15α 2-IR in the heart and further characterizing the role of cardioactive neurons in Aplysia.
IN-SITU HYBRIDIZATION WITH NON-RADIOACTIVE PROBES IN WHOLEMOUNTS OF APlysia GANGLIA. J.K., Pom-1 and R.G. McCann. Dept. of Biolog, Calif. State Univ., Fullerton, CA 92634 and Div. of Neurosciences, Beckman Research Inst. of the City of Hope, CA 91028.

The large identifiable neurons in the CNS of Aplysia californica may be useful for determining the subcellular expression of ion channels that have been cloned. We have developed in situ hybridization preparations of wholemounts of Aplysia ganglia to facilitate identification of specific neurons containing particular mRNA transcripts of interest. These neurons may later be used in live preparations to test for the presence of specific types of ion channels. CDNA probes to neuronal interest and FMRFamide, previously cloned from Aplysia, were labeled with digoxigenin.

Results with these probes for abundant mRNAs indicate that the staining with the digoxigenin-based method is similar in appearance to immunohistochemically treated wholemounts of ganglia. Additional studies are planned to determine whether this procedure has the sensitivity to detect rarer Aplysia mRNAs. (Supported by CSUF Mini-Grant and NIH AREA Grant to JK0 and Beckman Research Funds to REM).
541.11
Using indirect immunocytochemistry on whole-mount tissues, we constructed a neuronal map showing met-enkephalin-like immunoreactivity in the CNS of Lymnaea. A total of 31 neurons, distributed among all except the pleural ganglia and left pedal ganglion, were consistently stained. In control experiments, no specific staining was observed when the primary or secondary antibodies were omitted, or when non-immune serum replaced the primary antiserum. Presumptive staining of the primary antigens with met-enkephalin-like peptide (60 ng) eliminated specific staining. Among the immunoreactive neurons were two giant, well-characterized peptidergic neurons: VD1 and RPD2. Their identity was confirmed in double-labeling experiments which combined Lucifer yellow fills with immunocytochemistry. These cells are strongly electrically coupled and are known to be involved in cardiorespiratory and whole-body withdrawal behaviour. Since these neurons innervate the heart, we examined this target organ for the presence of met-enkephalin-like peptide and found that a met-enkephalin-like peptide is present in the heart extracts, which may have a functional role in cardiovascular control.
Supported by MRC (Canada).

541.13
A PECK OF PICKLED PEPTIDES. W. Lessar and M. J. Greenberg. Whitney Lab, St. Augustine, FL 32086.
Two series of cDNA clones have been sequenced from the garden snail Helix aspersa: clone HF1 and clone HF4. Clone HF1 encodes only the two tetrapeptides FMRFamide and FRFamide; clone HF4 has sequences for eight longer peptides but no tetrapeptides. The peptides can be identified and quantified in tissue extracts with HPLC and RIA, and their distributions within this particular tissue can be visualised with immunohistochemistry. The two tetrapeptides and seven of the longer peptides end -RFamide while the eighth peptide encoded in clone HF4 has the sequence pQDPFLRamide. HPLC/RIA analyses of heart extracts suggest that only the tetrapeptides are present in the heart. However, the heart stains with antisera against EFLRamide implying that pQDPFLRamide and the other longer peptides are also present. Are the tetrapeptides the only FMRFamide analogues present? In the heart? If so, then why does the EFLRamide antisera stain the heart? If the longer FMRFamide analogues are present in the heart why are they not detected by HPLC/RIA?
Funded by NIH grants P32 HL08371 and HL28440.

541.12
THE MECHANISM OF GLUTAMATE ACTION IN THE BUCCAL CGP OF HELISOMA TRIVOLVIS. K. M. Quinlan and A. D. Murphy. Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60607.
The buccal CGP of Helisoma is comprised of three subunits (S1, S2, S3). Glutamate activates both S1 and S2, and its agonist effect of S2 on identified S2 postysyncaptic neurons, suggesting that the S2 neurons are glutamatergic. It appears that inhibition of S2 plays a critical role in the temporal linkages among subunits. Neurons that receive inhibition from S2 are hyperpolarized by glutamate and quisquulate, but not ACPD. These hypofunctions are blocked by the AP4 insensitive metabotropic receptor. S2 excitation appears to be mediated by a kainate-like receptor, AMPA, glutamate and AMPA and kainate depolarized neurons that receive excitation from S2. S2-evoked EPSPs and depolarizations produced by glutamate, AMPA, and kainate were blocked by CNQX. Glutamate, kainate and AMPA also stimulated rhythmic S2 activity. If kainate was used to evoke rhythmic S2 activity, addition of CNQX inactivated S2. If S2 activity was evoked by other neurotrans-active substances, CNQX blocked the S2-evoked EPSPs but not the S2 IPSPs, demonstrating that rhythmic S2 activity can occur in the absence of kainate-like excitatory influences. (NIH ROI NS26145)

542.1
EFFECTS OF REPEATED RESTRAINT STRESS IN RATS ON NEUROENDOCRINE AND MOLECULAR MARKERS OF THE BRAIN'S RESPONSE. Yoshimi Watanabe, Jesus Angulo, G. Bing1, D. Filer1, E. A. Stone1 and B. S. McEwen Lab. Neurendocrinol. Developmental Neurobiology, NIH, Bethesda, MD 20892 and LBG, St. Elizabeth's Hospital, NJMH, Washington, DC 20032.
We have continued our studies (Neurosci. Abstr. 16:550) on the receptor for the invertebrate neuropeptide FMRFamide by using a radioligand to characterize the binding site and by using antibodies to solubilize the receptor. 125I-daYFNa binds to optic lobe membranes with a Kd of 0.15 nM and a Bmax of 2.5 pmol/mg membrane protein at the optimal pH of 6.5. Binding is displaced potently by FMRFa (IC50 = 10-10 M) and weakly by FMRF-100 (10 μM). GTP, GTP-γ S and GDP reduce binding by up to 85 % with IC50 = 5-10 μM while 100 μM ATP and GMP have no effect. FMRFa and β2 stimulate adenylyl cyclase in optic lobe membranes while β5 is a partial antagonist. Both non-ionic (Triton X-100, octyl glycoside) and ionic (CHAPS, Zwittergent 3-10) detergents selectively solubilize the receptor. FMRFamide (100 μM) inhibits labeling of the solubilized receptor by β2 and β5 while FMRF-100, serotonin, and leu-enkephalin (all at 100 μM) do not.

542.2
A chronic osmotic stimulus (12 days drinking 2% saline) can influence the hypothalamic-pituitary axis by inhibiting the secretion of basal and adrenocorticotropin (ACTH) in rats (1). To determine whether the stress response is affected, we measured plasma levels of ACTH, corticosterone and oxytocin in saline-treated rats during a 5 minute restraint stress. Mean plasma ACTH levels were markedly decreased from 972 ± 165 pg/ml in the control animals to 349 ± 114 pg/ml in animals given 2% saline. The stress-induced release of ACTH seen in the control rats (from 972 ± 165 to 1439 ± 105 pg/ml) was completely abolished in rats treated with 2% saline for 12 days (unstimulated 349 ± 114 pg/ml, stressed 205 ± 27 pg/ml). A stress-induced decrease of corticosterone was seen in control rats (from 28.8 ± 7.9 to 99.5 ± 8ng/ml) but not in saline-treated rats (stressed 36.0 ± 8.2ng/ml). A normal ACTH response to stress was obtained in adrenalectomized rats on isotonic saline (from 2383 ± 466 to 4065 ± 195pg/ml, n=9) while ACTH levels in both unstressed and stressed rats on 2% saline were reduced (1175 ± 213 and 2338 ± 369pg/ml respectively, n=9) while ACTH levels in both unstressed and stressed rats on 2% saline were reduced (1175 ± 213 and 2338 ± 369pg/ml respectively, n=9). In contrast to ACTH and corticosterone, the oxytocin response to stress was intact in animals on 2% saline. These results demonstrate that a chronic osmotic stimulus activates an inhibitory mechanism over the release of ACTH and its adrenal corticosterone and oxytocin responses to stress.

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452.3 CORTICOTROPIC-RELEASED HORMONE (CRH) IMMUNOREACTIVITY (IR) IN THE PARAVASCULAR NUCLEUS POMOLOLEMA (PVN). D.W. Elson, M. Del Bigio, T. A. Handa. Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University, Chicago, Maywood, IL 60153.

The plasma corticosterone response to novelty stress is greater in gonadectomized male rats. To further characterize the role of androgens in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis we castrated (gx'd) male Sprague/Dawley rats and treated them with testosterone propionate (TP). Control animals were castrated and sham treated or left intact. After 3-4 weeks of treatment, animals were perfused through the ascending aorta with paraformaldehyde, and CRH-positive (V) IR was examined in 20 m thick frontal sections. The number of CRH-IR cells in the bed nucleus of the stria terminalis was significantly greater by 68% in the PVN in gx'd animals compared to intact controls. T replacement restored CRH-IR cell numbers to that of intact animals. No differences were seen in VP-IR cells in the PVN or in CRH-IR cells in the bed nucleus of the stria terminalis. These data suggest that androgens can influence the HPA axis at the level of the PVN.

452.4 INDIVIDUAL DIFFERENCES IN THE HYPOTHALAMIC-PITUITARY-ADRENAL RESPONSE TO STRESS: RELATIONSHIP TO TESTOSTERONE. V. Vial & M. J. Meaney. Dept. of Pharmacology Research Ctr., Dept. of Psychology and Neurology/Neuropagey, McGill Univ., Montreal H3H 1R3, Canada.

Male rats typically show smaller responses to stress than females, but the underlying endocrine mechanisms are not well understood. To investigate whether variation in the hypothalamic-pituitary-adrenal (HPA) axis is related to individual differences in stress response, we compared T levels among male rats that responded to acute stress with high or low ACTH responses. Initial comparison of T levels revealed no significant differences between high and low responders. However, when the high responders were further divided into those that were castrated (GDX) and intact, the GDX group showed lower T levels compared to the intact animals. These findings suggest that T is a potent regulator of HPA activity during stress, as slight variations in T levels are associated with profound differences in the pattern of ACTH and B responses to stress. Thus, in the male rat, individual differences in HPA responses to stress are at least partially related to the gonadal status of the animal.

452.5 ABSENCE OF CORTISOL NEGATIVE FEEDBACK REGULATION OF PULSATILE ACTH IN THE OVINE FETUS NEAR TERM. L.M. Apostolakis, L.D. Longo, S.M. Yellen. Dowsing Biology, Deps. of Physiol, Pediat, Ob/Gyn, Loma Linda Univ, Sch of Med, Loma Linda, CA 92330

During late gestation, as a part of the endocrine cascade which triggers parturition, plasma cortisol increases in the fetal sheep to surge within 48h of birth. Flowing the hypothalamic-pituitary-adrenal (HPA) axis we castrated (GX'D) male Sprague/Dawley rats and treated them with testosterone propionate (TP). Control animals were castrated and sham treated or left intact. After 3-4 weeks of treatment, animals were perfused through the ascending aorta with paraformaldehyde, and CRH-positive (V) IR was examined in 20 m thick frontal sections. The number of CRH-IR cells in the bed nucleus of the stria terminalis was significantly greater by 68% in the PVN in GX'D animals compared to intact controls. T replacement restored CRH-IR cell numbers to that of intact animals. No differences were seen in VP-IR cells in the PVN or in CRH-IR cells in the bed nucleus of the stria terminalis. These data suggest that androgens can influence the HPA axis at the level of the PVN.
542.9  DEXAMETHASONE BLOCKADE DOES NOT ABOLISH ULTRADIAN RHYTHMS IN CORTICOSTERONE SECRETION BY THE RAT ADRENAL. M.S. Jasper and W.C. Engeland, Sect. of Neurobiology, Dept. of Surg., Brown Univ./RI Hospital, Providence, RI 02903.

An ultradian rhythm in corticosterone (B) secretion has been demonstrated in the rat using intracranial measurement systems (FASEB J. A4:271, 1990). To determine if this rhythm is independent of hypothalamic corticosterone secretion, rats were decapitated 10 seconds after injection. Six micron thick cryostat sections were cut from adrenal glands of restrained, male Long-Evans rats. The adrenal probe was constructed from cellulose fibers (9 kD MW cutoff). Experiments were conducted 2 days post-surgery. Sections were stained for alkaline phosphatase and rephotographed by light microscopy. Percent capillary volume and filling were calculated for each region. Volume percent filling averaged 75%, 75% and 86%. These results do not support the hypothesis that selective filling occurs in restricted microvascular components in B secretion. These data suggest that ultradian rhythmic variation in adrenal B secretion can occur in the absence of physiological variation in ACTH stimulation; however ACTH appears to modulate the amplitude of the B pulses. Supported in part by NIH grant DK38951.

542.11  INHIBITION OF PLASMA CORTICOSTERONE LEVELS BY THE SUPRACHIASMATIC NUCLEUS. R.M. Buijs
d, T.P. van der Woode, A. Kalsbeek and J.J. van den Hurk, Neuroendocrine Unit, Ottawa Civic Hospital, Ottawa, KYI 4E9, Netherlands Inst. for Brain Research, 1105 AZ Amsterdam.

The suprachiasmatic nucleus (SCN) is established as the (main) circadian clockwork of the mammalian brain. As such, it synchronizes a number of humoral and behavioral rhythms to the daily light-dark cycle, of which plasma corticosterone (B) for example, is shown to rise shortly before the onset of the dark period. A number of studies have shown that the SCN is involved in the regulation of this phenomenon. Recently, we provided evidence that vasopressin from the SCN inhibited B release when infused in the paraventricular-, and paraventricular-, and paraventricular, hypothalamic nucleus. To investigate further the role of the SCN (that nucleus was lesioned b) laterally, whereby the animals received an intraventricular bulbous jugular vein catheter allowing unrestricted blood sampling. Plasma B levels of SCN-lesioned animals during the light period were not different from those observed during the dark period. However, an increase of 10% was observed in the light period. No difference was observed in plasma B levels between SCN-lesioned animals and intact animals during the dark period. After receiving a mild stress the plasma B excursion in lesioned animals was much greater than that of intact animals during the light period but was comparable to that obtained from intact animals during the dark period. These data show that the SCN serves an inhibitory role on plasma B release but only so during the light period.


Neuroendocrine changes were, therefore, characterized in the shorter lived and hyper-reactive to stress Wistar Kyoto (WKY) strain and compared to the Brown Norway (BN) strain. We found: 1. catecholamines, similar in the young strains, were reduced (20%) and were less effective in restraining stress in aged WKY.  2. CRH binding, similar in the strains, was reduced (25%) in aging. 3. ACTH levels, 67% higher in WKY, were not changed in aging and both strains were similarly responsive to stress: 4. corticosterone, similar in the strains, was reduced (50%) in aging, but the stress-induced increase was relatively higher and was maintained longer compared to young. 5. prolactin, two fold higher in young WKY, was reduced (60%) in aged BN, but the stress response remained similar in both strains. The data support the premise that during aging regulatory neuroendocrine mechanisms deteriorate and the rate is genetically determined.

542.13  CAPILLARY VOLUME AND FILLING IN THE NEUROHYPOTHALAMUS AND HYPOTHALAMUS. R.E. PAGE, T. RUTHERFORD, and R. M. BRYAN. Division of Neurosurgery, College of Medicine, Pennsylvania State University, Hershey, PA. 17033.

We tested the hypothesis that selective filling occurs in restricted microvascular regions supplied by capillary loops in the median eminence by measuring percent capillary volume and filling in the median eminence and selected hypothalamic nuclei. 0.5 mL of FITC dextrans (150 mg/kg) was injected via a femoral venous catheter over 4 seconds into six restrained, awake, male Long-Evans rats. The animals were decapitated 10 seconds after injection. Six micron thick cryostat sections were taken through the median eminence, and paraventricular nuclei. Sections were examined and photographed by fluorescence microscopy. Stained for alkaline phosphatase and rephotographed by light microscopy. Percent capillary volume and filling were calculated for each region. In the median eminence, capillary volume averaged 28%. The internal plexus accounted for more than 50% of the total capillary volume. Plasma volume percent filling averaged 96% in the median eminence and 93% in its internal plexus. In the SON, PVN, & ARC capillary volume percent averaged 15%, 7% and 4% respectively. Capillary percent filling averaged 75%, 75% and 86%. These results do not support the hypothesis that selective recruitment of capillary loops occurs in the internal zone of the median eminence, but do allow the possibility of capillary recruitment in the Paraventricular Nucleus.


There is extensive evidence that the suprachiasmatic nucleus (SCN) contains the circadian oscillator responsible for 24-hour rhythms in many behavioral, endocrine, and autonomic functions. The mechanism by which information from the SCN is transmitted to other brain regions regulating these functions is unknown. Recently, by means of injection techniques, it was demonstrated that peptides present within projections of the SCN may be involved in the control of these daily rhythms, in particular that of plasma corticosterone levels. This rhythm is considered to involve parvocellular neurons in the hypothalamic paraventricular nucleus that secrete CRF into the median eminence (ME) portal circulation.

To further understand the way by which the SCN may impose rhythms on centrally controlled processes we undertook electrophysiological recordings from neurons situated in the region of the paraventricular hypothalamic nucleus in parietal bulbous anesthetized male Long-Evans rats and examined their response to electrical stimulation of the SCN. A total of 31 spontaneously active neurons, 4 could be identified as projecting to the ME. Following SCN-stimulation, peristimulus histograms of the ME-projecting neurons displayed a distinct 40-80 msec reduction in excitability. Of the 27 neurons that were not projecting to the ME, 23 were likewise depressed, one was stimulated and 3 were not responsive. The data suggest that in this experimental situation the SCN exerts a predominantly depressant influence on neurosecretory and other unidentified neurons in the area of the hypothalamic paraventricular nucleus. Supported by MRC and Heart and Stroke Foundation.
543.1 LHRH IS MORE POTENT THAN ENDOTHELIN-1 (ET-1) IN STIMULATING CHANGES IN INTRACELLULAR CALCIUM AND LH SECRETION FROM SINGLE PROESTROUS RAT GONADOTROPHS. S.R. Rawlings*, J. Flores*, L. Bloomfield, CO). The spikes were categorized by their various firing rates of single units in the mediobasal hypothalamus and the firing rate of single units in the MUA volleys. A low-dose of TTX. At high doses (100 nM) GnRH induced biphasic responses to ET-1 and LHRH were highly variable from cell to cell and showed response profiles typical of factors that stimulate an Ins(1,4,5)P3 and DAG generating system. LHRH was, however, more potent at stimulating Ca2+ responses as summarised below:

**Ca2+ Response**
- **LHRH**
  - **No response**
  - **Low amplitude rise**
  - **Ca2+ oscillation**

- **ET-1**
  - **ET-1**
  - **Ca2+ response**
  - **ET-1**
  - **Ca2+ oscillation**

- **Spontaneous**
  - **Spontaneous**
  - **Ca2+ response**
  - **Spontaneous**
  - **Ca2+ oscillation**

In the RHPA the ED50 for LHRH and ET-1-stimulated plaque number (a measure of gonadotropin secretion responsiveness) was 10 pM and 3 nM respectively. In conclusion, the potency of ET-1 to stimulate proestrous gonadotropes is significantly lower than the known regulator of gonadotrope function LHRH, calling into question any role of ET-1 in the mire regulation of LH release during the estrous cycle.


Pituitary gonadotropes secrete LH and FSH in response to GnRH, which induces release of Ca2+ from intracellular stores and oscillations of [Ca2+]i. This process and the regulation of voltage-activated Ca2+ channels are considered to be involved in the control of GnRH-induced gonadotropin secretion. Basal and receptor-regulated changes in [Ca2+]i and membrane potential were monitored by fluorescence analysis and the perforated patch technique in individual rat pituitary gonadotropes. All measurements were conducted at 20°C, 95% O2. Most gonadotropes exhibited episodic spontaneous oscillations in basal [Ca2+]i, that were abolished by addition of EGTA or Ca2+ channel blockers. Under control conditions, gonadotropes also displayed continuous electrical activity with firing of action potentials (AP) at a frequency of 0.6 to 1.6 Hz. These AP did not overshoot and were insensitive to 15 μM TTX. At high doses (100 nM) GnRH induced biphasic response in [Ca2+]i, with a spike phase, followed by a oscillatory phase. Addition of 100 nM GnRH to the bath resulted in hyperpolarization from -35 mV to approximately -70 mV and inhibition of AP, followed by a pattern of regular, slower oscillations (0.1-0.3 Hz) of Vm between -65 and -35 mV. AP were regularly fired on top of the slow waves. However, in some cells slow oscillations occurred without AP firing, suggesting that both phenomena are independently modulated. Slow oscillations were insensitive to 20 mM TEA and were broadened by 1 μM BK 8644. These observations suggest synchronization of [Ca2+]i, and electrical activity in pituitary cells. Modulation of a Ca2+-activated current and plasma membrane Ca2+ channels may be involved in GnRH-evoked Vm oscillations.


Secretory pulses of gonadotropins occur synchronously with sharp increases in MUA (volleys) recorded from the medio basal hypothalamus (Neuroendocrinology 39:256, 1984). Such recordings from 4 unanesthetized ovariectomized monkeys were digitized and analyzed for their component spikes using cluster analysis (Discovery Software - Brainwave System Corporation, Bloomfield, CO). The spikes were categorized by their various dimensions (principal components) and firing frequencies compared to those of the MUA. Of the 22 individual spike clusters identified, 15 increased and 2 decreased their firing rates synchronously with the MUA volleys. The firing rate of the remainder was unchanged. No evidence of recruitment was found. It is concluded that the MUA volleys that reflect the activity of the GnRH pulse generator represent the synchronized increase in firing rate of single units in the medio basal hypothalamus and the simultaneous decrease in the firing rate of a smaller population of others. (Supported by NIH grants HD 17438 and HD 08610 and by The Clayton Foundation for Research)

543.5 HYPOTHALAMIC ACTIONS OF C-TYPE Natriuretic Peptide (CNP) TO ALTER PITUITARY HORMONE SECRETION. M. K. Hamson and F. R. Huynh. Anatomy/Neurobiology, Univ. MD Sch. of Med, Columbia, MO 65212.

C-type natriuretic peptide (CNP) shares a high degree of homology with ANP and BNP. CNP is present in brain in higher levels than ANP or BNP and binds preferentially to the ANP/BNP (ANP-BNP) receptors. We compared the effects of CNP and ANP on the hypothalamic control of LH and PRL secretion. Like ANP, CNP failed to alter LH, PRL or GH release from cultured, dispersed anterior pituitary cells. Both CNP and ANP significantly inhibited LH secretion when administered centrally; however, the minimum effective dose of CNP was 0.1 n mole, while 1.0 n mole of ANP is required. The inhibitory effect of CNP and ANP was abolished by prior, central injection of the delta opioid antagonist naltrindole. While central injection of ANP inhibited PRL secretion, 1.0 n mole CNP stimulated (P<0.001) PRL secretion in conscious rats. Plasma GH levels were not significantly altered. The data suggest that CNP might be the preferential neuroactive natriuretic peptide in brain and that endogenous CNP might exert physiologic effects on pituitary function.

543.6 Dopamine CONTENT IN THE BRAIN AND PITUITARY OF TILAPIA (Oreochromis mossambicus). S. Ishwar*, and K. S. Kim*. Lab of Neurobiology, Rockefeller University, New York, NY 10021 and Dept. of Biology and Pharmacology, National University Singapore, Singapore.

Hypothalamic dopamine (DA) has been shown to participate in neuroneuroendocrine regulation of gonadotropin release in fish (Peter et al., Proc. Soc. Biol. Med. 42:513, 1980) and DA turnover in hypothalamus of the cat is decreased by sex steroids. In the present study we used HPLC with electrochemical detection to examine the relationship between sex steroids and ex vivo brain and pituitary DA and DOPAC levels in tilapia. Tissue was taken from intact, gonadectomized (16d) and castrated (16d) with testosterone (T) or estradiol (E2) replacement on days 14 and 17 (n=4). In intact fish, DA and the DA/DOPAC ratio, tended to be highest in the pituitary and the tibia followed by hypothalamus. Although not statistically significant, there was a tendency for T to decrease, and E2 to increase, DA levels in all brain regions (telencephalon, tuberal area, lateral lobes, optic tectum and thalamus) and the pituitary.

In a subsequent experiment, electrolytic lesions were made stereotactically (Ishwar and Munro, unpub.) in the hypothalamus of Tilapia (4-5 cm) to induce permanent amenorrhea and laparotomy and gonadectomy. Relative to controls, animals with pretreatment lesions (16d) had significantly higher DA levels in the pituitary. Lesions in other areas of the hypothalamus had no effect. Thus this experiment provides evidence for the existence of a preoptic-hypothalamic neural system that inhibits pituitary dopamine in tilapia.
543.4

DUAL MECHANISMS INVOLVED IN THE INHIBITORY INFLUENCE OF SUCKLING ON LH RELEASE IN THE OVARIECTOMIZED RAT. T.-J. Wu, N.J. McDonald. Departments of Psychology and Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77843.

A study was conducted to further understand the involvement of the endogenous opioid peptides (EOPs) in suckling-inhibited induction of LH release in the rat. The experiments utilized postpartum rats (day 7) that had been ovariectomized the day following parturition. The first experiment was designed to determine the effect of opioid antagonism by naloxone (N L), 0.1 mg/kg, on the increase in peripheral LH concentration after 18 h of pup removal and the decrease in LH concentration after 18 h of resuckling. NAL during 18 h of pup removal or during 18 h of resuckling by 18 h of resuckling neither accentuated nor attenuated (p > 0.10) the LH concentration. The second experiment was designed to determine the effect of NAL on peripheral LH concentration in continuously suckled rats. Serum LH increased (p < 0.01 and p < 0.005, respectively) in response to 18- and 36-h N AL. Therefore, the effects of pup removal by 18 h of resuckling neither accentuated nor attenuated the LH concentration. These results suggest that suckling may inhibit LH release through two mechanisms. The first may be an opioid-independent or enhanced opioid tone, important for the initiation of the inhibitory effect of suckling, and the second, an opioid-mediated mechanism for the sustained inhibitory effect of suckling on LH release.

543.5

NEURONAL HYPERTROPHY IN THE HYPOTHALAMUS OF OLDER MEN. N.E. Rance and N.T. McFall. Departments of Pathology and Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724. A striking neuronal hypertrophy occurs in the infundibular nucleus of postmenopausal women (Rance et al, J Clin Endo Metab, 1990; Rance and Young, Neurosci. 1991). To determine the greater specificity of this response, we measured cross-sectional areas in the infundibular hypothalamic nuclei of young (24, 32, and 41 yr of age) and older (60, 61, and 68 yr) men and women and compared them to data reported previously from the hypothalami of pre (28, 32, and 40 yr) and postmenopausal women (58, 62, and 74 yr). Formalin fixated hypothalami were paraffin embedded, serially sectioned and quantified. Cross-sectional areas of 2427 neurons were traced using an image-processing microscope. The mean cross-sectional area (SEM) of neurons in the infundibular nucleus of young men (147.0 ± 1.3) was identical to that of young women (147.0 ± 2.2). The mean area of infundibular neurons in postmenopausal men (186.1 ± 2.3) and women (188.4 ± 2.3) was 30% greater than those from young men and women. We were intrigued to find that infundibular neurons from older men (171.9 ± 2.7) were significantly larger than those from young men and women, but still smaller than infundibular neurons from postmenopausal women. We have previously provided evidence that postmenopausal neuronal hypertrophy is secondary to the loss of ovarian steroids (Rance et al, J Clin Endo Metab, 1996; Rance and Young, 1991). The moderate hypertrophy of infundibular neurons in older men may be due to the reduced circulating testosterone levels which have been reported in this age group. (Arizona Disease Researchr Commission 1990-0299)
GLUCOCORTICOID AND ESTROGEN INHIBITION OF CRF INDUCTION OF POMC GENE EXPRESSION. S. J. Dermer, D. L. Lorens, and L. J. Roberts. Fishberg Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

Glucocorticoids and estradiol inhibit expression of the POMC gene via steroid receptors-mediated events. In particular, corticosterone, glucocorticoids inhibit CRF transcription directly and by interfering with transcriptional induction by GRF in the hypothalamus. These two hormones have direct inhibitory effects on POMC transcription. We have focused on comparing the mechanisms by which the two steroid hormones and their respective receptors influence expression of the POMC gene. Using a variant (E-1) of the A2T2 D16-16 mouse anterior pituitary tumor-derived cell line, which expresses functional short term estrogen (E2) treatment (25 or 30 min) (100nM) inhibits the stimulatory effects of CRF treatment (10nM) on POMC primary transcript and hRNA levels. This effect is found using pretreatment with either CRF or E2 followed by short term cotreatment, and when the two hormones are added simultaneously. In A2T2 cells, using a similar experimental paradigm for glucocorticoids and CRF cotreatment, we find that pretreatment with DEX inhibits CRF induction of hRNA levels, whereas pretreatment with CRF followed by cotreatment does not affect CRF induction. When a transgenic transactivator, a POMC promoter/enhancer (304-38)-CAT fusion reporter gene into ER-1 cells and treat with E2 (16 hr), we find that CRF induction of the POMC promoter is diminished versus cells which are treated with CRF alone. Similarly, in DEX treatment (100nM) of ER-1 cells also leads to a reduction in CRF induction of the POMC-CAT reporter. These results, along with our previous findings that estrogens and glucocorticoids do not elicit the same response in POMC primary transcript for acute treatment times suggest that the two hormones may use different mechanisms of regulation of POMC gene expression initially, but their long term overall effects are inhibitory.

ENDOGENOUS OVARIAN STEROID(S) MAINTAIN IMMUNOCYTOCHEMICAL NUCLEAR LOCALIZATION OF GLUCOCORTICOID RECEPTOR IN ADRENALACTOMIZED FEMALE RAT BRAIN. W. C. McGimsey, J. A. Cidlowski, W. E. Stumpf, M. Sart. Departments of Cell Biology and Anatomy, and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

We have shown with the anti-glucocorticoid receptor antibody GR-57 nuclear localization of glucocorticoid receptor immunoreactivity (GR-ir) in brain areas of intact male rats, and adrenalectomized (ADX) male rats treated with corticosterone, dexamethasone, or RU486, and cytoplasmic GR-ir in brains of adrenalectomized male rats (McGimsey et al., Endocrinology 126:1484 1990; FASEB J 5:2835 1991). Since progesterone is known to bind GR this study tests the hypothesis that endogenous ovarian steroid(s) cause nuclear localization of GR-ir in ADX adult female rats. Vitrabone sections of paraformaldehyde fixed brains from intact, adrenalectomized (ADX), and adrenalectomized-ovariectomized (ADX-OVX) adult female rats were incubated in GR-57 (1:10,000) for immunocytochemistry. GR-ir was nuclear in intact and ADX female rats' brains, and cytoplasmic in ADX-OVX rats' brains. Supported by USPHS Grant NS-17479.
544.4


Acute food deprivation during estrus cycle Days 1-2 will block hamster ovulation (Morn, AAJP, 251:R663, 1986). This is precipitated by retarded follicular development, low peripheral estradiol levels, elevated LH, decreased ovulation, and loss of ovulatory response to exogenous estradiol or LH. The present three studies further elucidate the endocrine deficits in the phasically starved hamster.

A) Starved animals were injected with 5 μg estradiol benzoate or oil 8 h before lights off on cycle Day 3; fed animals received oil. Fed controls ovulated; starved controls did not. Starved females had low LH levels throughout the cycle, including Day 4 when the ovulation surge was seen in fed females. Variability in LH measured during Days 2 and 3 tended to be greater for starved animals. C) LH was measured for 2 h periods at 8 mm intervals during the afternoon of Days 1 and 2 or during the morning of Day 2. Pulsaive LH was not abolished in animals that failed to ovulate in response to acute starvation.

The results confirm the absence of an ovulatory LH surge in phasically starved hamsters and show that estradiol priming will reinstate the surge without restoring ovulation. This is probably explained by the retarded follicular development in the food deprived animals. The precipitating endocrine events preventing normal follicle growth remain obscure.

Supported by MH42684 to LPM.
454.9
STIMULATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) RELEASE FROM HYPOTHALAMIC EXPLANTS OF MONKEYS (MACAQUE BOB) 13 IS BLOCKED BY beta-ENDORPHIN. Richard W. Clough, Dept. of anatomy, Southern Illinois Univ. School of Medicine-Carbondale, Carbondale, IL 62901

GABA has stimulatory and inhibitory effects on luteinizing hormone (LH) secretion in male and female animals, and the direction of the effect presumably reflects a site specificity in the brain (Olson, Science 186,1979). In vitro studies demonstrate that GABA stimulates LH release from arcuate nucleus-related fragments (Masato and Nuevo-Vilar, GABA and Endocrine Function. Raven Press, NY, 1986). We have confirmed that 544.11 NAL stimulates LH release from propic area-medial hypothalamic (POA-MBH) explants in perfusion tissue culture (Clough et al., Neuroendocrinology, 51, 1990). The similarity between the effects of GABA and NAL led us to investigate whether the stimulatory GABA effect may involve the opioid system. POA-MBH explants were removed and placed in culture as previously described (Clough et al.; Brain Res, 444,1988).

Following equilibration, POA-MBH explants were exposed to culture media containing either: GABA (2X10^-4 M); beta-endorphin (1.4 X 10^-7 M), or a combination of the two drugs. Subsequent to drug challenge, explants were exposed to KCl (57mM) for viability assessment. Exposure of explants to GABA resulted in a significantly increased LH release. Beta-endorphin had no effect on basal release. However, exposure of explants to combined GABA and beta-endorphin completely abolished the stimulatory effects of GABA on LH release. In all experiments, a final KCl challenge effectively stimulated LH release. This study suggests that the stimulatory effect of GABA on hypothalamic LH release, and presumably stimulation of pituitary gland LH, is mediated by a series of opioid beta-endorphin and subsequent release of tonic inhibition (as seen with NAL). Supported by NIH HD24426.

454.10

These studies examined the effects of naloxone (NX) or morphine (MOR) on LH responsiveness to NPY (544.11 45 μg) alone or following electrophysiological stimulation (ECS) of the medial preoptic area (MPOA) in ovariectomized, estrogen-treated rats. Pretreatment with NX (5 mg/kg at -15 min) neither suppressed nor enhanced the release of LH evoked by NPY. 45 μg at 0 min) or combined MPOA-ECS + NE. Thus, the opiate synapses which exist on LHRH neurons do not seem to modulate neural responsiveness to NE but may activate the preprotachykinin-like release of NE. In contrast, when MOR (20 mg/kg) was injected 45 min prior to ICV NE, LH release more than doubled (245-450 ng/ml). When -15 min) was combined with MPOA-ECS (0 min) + NE (30 min) peak LH values increased from 200 (NE only) to 1600 ng/ml. MOR blocks spontaneous LH surges and hypothalamic NE increase and markedly decreases prolactin secretion, an observation confirmed in these studies. This latter effect has been attributed to increased secretion (5-HT) which in turn, may suppress dopamine (DA) release. To test this possibility, in other rats, MOR (10 μg/7 ul) was injected into the dorsal raphe nucleus (DRN) (-15 min) and ICV NE was given at 30 min. Amplified LH responses were equivalent to those obtained after ICV NE. Neither DRN-MOR nor saline alone altered basal LH secretion. Combined DRN-MOR + MPOA-ECS + NE resulted in a fall in plasma LH (peak surge values). Thus, the combined priming of LHRH neurones with 5-HT (or suppression of DA) coupled with the removal of inhibitory GABA influences (by MPOA-ECS) and stimulation of NE may be important endogenous events which lead to LH surges. None of these treatments alone can produce this heightened LH neuronal response. Supported by HD-92138.

454.11
NEUROPEPTIDE Y (NPY) POTENTIATION OF LHRH-INDUCED LH SECRETION IS STEROID-DEPENDENT. J.L. Levine and A.C. Rauw-Daneld. Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

We recently demonstrated that NPY potentiates the ability of pulsatile LHRH infusions to restore LH surges in perchorbitally-implanted, propranolol-treated rats. The present study tested the hypothesis that facilitatory actions of NPY occur during ovarian steroid-induced LH surges and not in unstimulated (OVX) animals. At 0900h on day 6 after OVX, female rats received 30 μg estradiol benzoate (EB) or oil sc, and 2d later received 5 mg progesterone (P) or oil sc. Hourly blood samples were obtained between 1100-2000h on the day of ova. Injections of PB to block endogenous LHRH release, or saline. Every 15 minutes, PB-treated rats received 10 pulses of LHRH (50ng/pulse) or saline, along with concurrent pulses of NPY (1 or 5μg/pulse) or saline. Pulsatile LHRH infusions restored LH surges in EB+P treated rats. The same dose of NPY was only marginally effective in rats treated with EB alone and completely ineffective in altering LH-induced LH secretion in oil-treated rats. These results demonstrate that the potentiation of LHRH-induced LH secretion in vivo is steroid-dependent and provide further support for the idea that the pituitary actions of NPY are specifically important for the generation of LH surges. (NIH HD26077)

454.12

Progesterone (P) enhances the activation of GnRH neurons during estradiol (E)-induced LH surge, and together with P stimulates the expression of the proto-oncogene product fos in the GnRH cells. Since GnRH neurons lack P receptors we investigated whether the P influence GnRH cells via neural intermediaries. The present study tests the hypothesis that the influence of P on GnRH neurons is mediated at least in part by noradrenergic receptor mechanisms. Ovx rats were pruned with P implants followed by an injection of P on day 2. After four hours, rats received an injection of the α-adrenergic receptor blocker, phenoxynbenzamine (POB, 2mg/kg i.v.), and were perfusion-fixed 2 hours later. Treatment with POB resulted in significantly lower peak LH levels as measured with RIA and in a dramatic reduction in the number of GnRH cells which showed fos immunoreactivity when compared to control rats. These data indicate that the enhancement of GnRH neuron activity by P was blocked by POB, and suggest that α-adrenergic receptor mechanisms are involved in the LH surge. (Support: WSU BMS Res. Grant and NIH HD24697).

454.13
ALPHA-1 ADRENERGIC REGULATION OF ESTROGEN-INDUCED INCREASES IN LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) mRNA AND RELEASE. G.D. Wensm, L.C. Kre, and D.W. Pfaff, Laboratory of Neuroendocrinology and Behavior, The Rockefeller University, New York, NY 10012.

Prazosin, an α-1 adrenergic antagonist, was used to examine the relationship between adrenergic inputs and the stimulatory effects of estrogen on LHRH mRNA and release. Bilateral cannulae were implanted just dorsal to the preoptic area (POA). Estrous cycles were monitored daily by vaginal smears. On the morning of proestrus, one rat was ovxtracised and assigned to one of three treatment groups: Destroy - injected with saline; Injected - injected with estradiol benzoate (EB, 10 μg) to produce an LH surge; or, Surprisingly - injected with EB and a prazosin-filled inner cannula was put into the POA. Between 4-6 pm of the following day, rats were anesthetized, decapitated, trunk blood collected, and brain were removed in liquid nitrogen. In situ hybridization was performed using a 32P-end-labeled 59-mer complementary to LHRH mRNA. Reduced silver grains, proportional to LHRH mRNA content, were quantified. Treatment with estrogen alone resulted in a 50% increase (P<0.05) in numbers of cells expressing LHRH mRNA. Reduced silver grains, proportional to LHRH mRNA content, were quantified. Treatment with estrogen alone resulted in a 50% increase (P<0.05) in numbers of cells expressing LHRH mRNA. Estradiol alone increased was blocked, EB injected (P<0.05) LHRH gene expression by 55%. Estradiol completely blocked (P<0.01) the estrogen-induced increase. Finally, the estrogen-induced LH surge was blocked by the local administration of prazosin. Thus, coincident with the LH surge, estrogen increased the number of LHRH-expressing neurones as well as total potential LH surge reactivity. KA Nlxotropic to the alpha-1 receptor may constitute a mechanism that enhances the ability of estrogen to increase LHRH mRNA and LH release. Therefore, it appears that an endogenous α-1 ligand, probably norpseudine, helps mediate the stimulatory effects of estrogen on hypothalamic release and release.
545.1

INTESTINAL DYSTENSION ACTIVATES A VAGO-VAGAL REFLEX TO ALTER WATER ABSORPTION BY RAT JUINUNIN VIVO. S. Zhang, K. Ohara, I. Carson, W. R. Rendall, R. Fogel. Henry Ford Hospital, Detroit, MI.

Little is known about the central pathways that transmit information about intestinal stimuli or how intestinal afferents regulate gastrointestinal functions. The aim of this study was to determine whether 1) stimulation of vagal mechanoreceptors by intestinal distention alters water absorption along the rat jejunum and 2) the role of the dorsal motor nucleus of the vagus (DMNV) in the mediation of this effect. Intestinal water absorption was measured in two segments of intestine. One loop was distended and the other was left intact, these loops were isolated separately from the rest of the gut, and the isolated loops were placed in a water bath. We found that 37 of 44 DMNV neurons were inhibited by distention. These results indicate that intestinal distention decreases the activity of neurons in the DMNV, and that inhibition of DMNV activity is responsible for increased absorption in the small intestine.

545.3

IMMobilIZATION-INDUCED HYPOCALCEMIA IS MEDIATED BY GASTRIC VAGAL ACTIVATION: THE STOMACH AS THE MAIN CALCIUM REGULATOR. R. C. Rogers. Ohio State University, Dept. of Physiology, Columbus, OH 43210.

Abdominal immobilization (IMB) group in which rats were tied their four limbs on the board. The rats were randomly divided into two groups: one with body weight). The rats were randomly divided into two groups: one with IMB and the other control. Intestinal distention did not increase water absorption in the control group when sympathetic ganglia were intact. However, distention increased absorption in the control loop if the sympathetic nerves were cut. Truncal vagotomy or selective vagotomy prevented the distention effect. Our results suggest that vagal afferents, terminating in the brainstem, initiate a reflex involving vagal efferents. To further explore this phenomenon we used glass micropipettes filled with 3.0 M KCl to record the activity of DMNV neurons while distending a segment of the jejunum. We found that 37 of 44 DMNV neurons were inhibited by distention. These results indicate that intestinal distention decreases the activity of neurons in the DMNV, and that inhibition of DMNV activity is responsible for increased absorption in the small intestine.

545.5

FUNCTIONAL ZONATION OF RESPONSES TO ANTRAL MECHANORECEPTOR ACTIVATION IN THE DORSAL VAGAL COMPLEX (DVC) OF RATS. Konrad C. Hohen. Ohio State University, Dept. of Physiology, Columbus, OH 43210.

Activation of gastric vagal afferents (by antral distention or electrical stimulation of the vagus) either excites (ON) or inhibits (OFF) the firing rate of cells in the DVC. In our extracellular recordings, ON cells were located dorsal to the OFF cells. The average depth from the dorsal surface of the brainstem was 534±55 and 624±14 μm in ON and OFF cells, respectively (p<0.001). This, together with histological evidence, suggested that ON and OFF cells have different functions. i.e., ON cells may be NTS neurons, whereas OFF cells could be DMN neurons. We studied this hypothesis directly by using electrophysiological criteria to identify distention-related responses as NTS or DMN neurons. Our results support the hypothesis that NTS and OFF cells can be functions of populations of neurons. Almost all ON cells (94%) are NTS cells, whereas many OFF cells (64%) are DMN neurons. These findings suggest further insights into the central organization of gastric vagal vago-vagal inhibitory reflexes. Supported by NS08690 (MKJ) and NS24530 (RCH).
545.7 CHEMICAL CODING IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS FOR GASTROINTESTINAL FUNCTIONS? S.L. Cohn, J.H. Wrenn, Jr., Lab. of Regulatory Physiology, Purdue University, W. Lafayette, IN 47907.

The variety of receptors in the dorsal motor nucleus of the vagus (DMNX) suggests that the control of gastric motility and secretion might be achieved at least in part by chemical coding or addressing. Two such identified receptors, those for thyrotropin-releasing hormone (TRH) and angiotensin II (ATII), were chosen to test the hypothesis that multiple-barelled pipettes were used to pressure inject, in random order, large volumes (50 nl) of a 1:1 solution of each of these peptides in the DMNX of TRH (1mM), ANG II (1mM), and saline into the DMNX of 16 rats (the same site/animal) while gastric secretion and motility were simultaneously measured.

As has been previously observed by others, TRH significantly increased acid output and gastric and duodenal motility. Further, TRH increased jejunal-ileal colonic activity. In contrast, ANG II did not activate either gastric secretion or motility, while it did significantly increase duodenal and motility. Electrical stimulation of the cephalic vagus elicited responses from all locations. The different patterns of GI activation produced by the peptides suggest that at least some of the TRH responses are mediated by the gastric subnuclei of the DMNX and their corresponding peripheral branches of the vagus, whereas, conversely, some of all the ANG II responses are mediated by the colonic subnuclei of the DMNX and their corresponding peripheral branches. Supported by NIH grants DK-27027 and NS-26032.

545.8 PANCREATIC NEURONAL CIRCUITS DEFINED BY TRANSENUCLEAR TRANSPORT OF PSEUDORABIES VIRUS. C. S. Sander, R. De Camilli, R. W. De Camilli, N. P. Card and L. Rinnaman. CURE and Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90073, Medical College of Pennsylvania, Philadelphia, PA 19104 and DuPont Merck Pharmaceutical Co., Wilmington, DE 19880.

Transneuronal transport of pseudorabies virus (PRV) was used to define the organization of neuronal network controlling pancreatic functions. Injection of PRV into the duodenum or splenic region of the rat pancreas identified a neuronal circuit including spinalafferent neurons, autonomic motoneurons and pancreatic efferent neurons. Injection of PRV into the pancreatic parenchyma revealed that spinalafferent neurons innervating the pancreas contained calcium gene-related peptide, visualized a synaptically linked circuit of central neurons within the neuraxis involved in vasculare function regulation and 3 identified a population of sympathetic preganglionic neurons in the intermediolateral cell column of the thoracic cord that project to postganglionic neurons in abdominal ganglia innervating the pancreas. Simultaneous injection of PRV and the amines of cholecystokinin-peptidelike connections revealed by pseudorabies virus transport. These findings confirm the specific uptake and transneuronal passage of a virus and define the neuronal circuits that contribute to the control of pancreatic function. Supported by NIH grant DK-87527 and DuPont Merck Pharmaceutical Co.

545.9 SEROTONERGIC MODULATION OF REPETITIVE FIRING ACTIVITY IN DORSAL MOTOR NUCLEUS NEURONS STUDIED IN VITRO. C. McNall and M. S. Deja, School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225. A brainstem slice preparation from adult guinea pigs was used to study both thalamic outflow pathways involving nerve terminals and 5HTI receptors in the DMNX of guinea pig neurons. Labeled DMNX neurons had large, ovoid cell bodies (30-40 um) and possessed several primary dendrites. Numerous serotonergic aspiny varicosities were observed in close apposition to cell bodies, while few appeared in the dendritic fields. Depolarization, DMNX neurons exhibited a high frequency burst of action potentials which rapidly adapted with time, i.e., spike frequency adaptation (SFA). A large after-hyperpolarization (AHP) was observed at the end of the depolarization. In the presence of 5HT1A receptor antagonists, a large AHP was reduced in a concentration-dependent manner (maximum response at 50 um). 5HT1A receptors mediated a large AHP that blocked the addition of 1 mM Ca2+ to the bath solution that a calcium-activated potassium conductance (IKC) mediated these properties. In 100 um, a selective antagonist for low conductance (LK) type IKC channels, completely blocked the AHP and the majority of the LK type IKC channels had no effect on either SFA or the AHP. Both SFA and the AHP were blocked by the addition of 1 mM Ca2+ to the bath solution. In the presence of 5HT1 receptors, the LK type IKC channels had no effect on either SFA or the AHP. In the presence of both apamin and 5HT1, DMNX neurons exhibited voltage-dependent conditional bursting activity. These data suggest that (1) 5HT1 modulated a voltage-dependent inward current localized to the soma of DMNX neurons and (2) this inward current could effectively compete with the LK type IKC channels which normally limit the repetitive firing activity of these neurons. (Supported by NIH grants HL40908, HL02514 and R307114).

545.11 C-FOS IS EXPRESSED IN ANATOMICALLY DISTINCT BRAIN NUCLEI FOLLOWING INHIBITION OR POTENTIATION OF FOOD INTAKE AND GASTRIC MOTILITY IN RATS. R.L. Gooze, G.H. Hoffman, A. Sved, E.M. Stieglitz, J.G. Viguier, Departments of Medicine, Physiology and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Most treatments that inhibit food intake (FI) and gastric motility (GM) in the rat result in activation of central oxytocinergic (OT) pathways, one of which projects to the dorsal motor nucleus of the vagus (DMNX) and the nuclius raphe obscurus (NRO). The present study was designed to determine the afferent pathways to the region of the NRO controlling GI function by using WGA-apoHRP-Au in Sprague-Dawley rats. Double-barelled micropipettes were filled with WGA-apoHRP-Au (1% solution) and L-glutamate (500mM) and inserted into the NRO in anesthetized rats with strain gauges attached to the stomach. Results. In two rats, L-glutamate microinjection increased the Minitrux index (+2.0 and +5.5) prior to WGA-apoHRP-Au (10 and 20nl microinjection). Four additional rats received 10 nl WGA-apoHRP-Au into the same region of the NRO. Numerous retrogradely labelled cells were located in the central medullary raphe nucleus, of which some were also serotonin-immunoreactive (ir) cells, and in the A1 noradrenergic group, of which some were also tyrosine hydroxylase-ir cells. Retrogradely labelled cells were located in the dorsal hypohalomic nucleus, the region above the ventral tegmental area, and the dorsal pons. Few retrogradely labelled cells were noted in the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis and central amygdala. Conclusions. Medullar projections to the NRO are similar to those projecting to the dorsal vagal complex, whereas supra-medulляр projections to the NRO differ substantially from those known to project to the dorsal vagal complex. These latter are potentially new pathways mediating control of GI function in anesthetized rats. Supported by the PMAF and an Institutional BRSG.

545.12 PARAVESICAL NUCLEUS STIMULATION-INDUCED GASTRIC DAMAGE IN HYPOPHYSECTOMIZED RATS. E.J. Sillery, J.L. Wallace, A.Y. Ferguson, Dept. of Physiology, Queen's University, Kingston, ONT, K7L 3N6, and Dept. of Medical Physiology, University of Calgary, Calgary, ALTA T2N 4N1.

Electrical stimulation in the paraventricular nucleus (PVN) of the hypothalamus has been shown to induce gastric mucosal lesions in the rat (Ferguson et al. 1988). Such damage is dependent upon stimulation parameters. The present study was designed to determine whether pharyngeal vagal afferent stimulation in isolation does not induce any significant gastric damage. Considering the well-established role of the PVN in the control of pharyngeal secretion, the present study was designed to determine whether pharyngeal vagal afferent stimulation can induce gastric damage in vivo. The present study was designed to determine whether pharyngeal vagal afferent stimulation can induce gastric damage in vivo. The results of the present study suggest that pharyngeal vagal afferent stimulation can induce gastric damage, whereas pharyngeal vagal afferent stimulation cannot induce gastric damage, whereas pharyngeal vagal afferent stimulation can induce gastric damage, whereas pharyngeal vagal afferent stimulation cannot induce gastric damage, whereas pharyngeal vagal afferent stimulation cannot induce gastric damage.

We have examined the functional consequences of producing an attenuated strain of pseudobacterial virus (Bartha) to define the visceral neuraxis innervating the esophagus, stomach and colon. In young adult rats the forebrain-to-cord extent of the central visceral neuraxis was labeled in a projection specific fashion. The labeling in the vagal complex at short survival times occurred in a motor viscerotopic pattern as seen with conventional tracers. At longer survival times the virus passed trans neurally to label putative 2nd order neurons in the 1)intermediolateral cell column, nucleus of solitary tract and area postrema 2)A, Aδ, and caudal caphe neurons, 3)juxtaocular area of hypothalamus, 4)paraventricular hypothalamic nucleus, 5)circular nucleus of amygdala, 6)bed nucleus of stria terminalis, 7)insular cortex, 8)2nd or 3rd order passage to LGN, VPL and nucleus medius, preoptic area, anterior and lateral hypothalamus, septum 9)3rd order passage to parabrachial nucleus and locus coeruleus. The pattern of viral replication in these regions was in a projection specific as seen with conventional tracers. This provides highly defined visceral neuraxis directly related to specific visceral organs and ties the circumventricular organs into it.

546.1 TEMPOROMANDIBULAR JOINT NOCICEPTORS RESPONDING TO HORIZONTAL AND VERTICAL DISPLACEMENTS OF THE MANDBLE OF THE GOAT. B. Loughner* and B. Cooper.


Experiments were undertaken to characterize nociceptors of the temporomandibular joint (TMJ). TMJ afferents were isolated from the trigeminal ganglion with tungsten microelectrodes. Force-monitoring curves indicated that capsular nociceptors responded exclusively to intense forces that produced extreme displacements of the mandible. Thirty-four units with receptive fields on the TMJ capsule were studied. Nineteen afferents responded preferentially to movement in the vertical plane (VP or opening) and 15 responded preferentially to movement in the horizontal plane (HP or lateral displacement). Once preferred movements were determined, tests of dynamic and static reactivity were conducted. Simple linear regression was used to construct power functions of either an exponential force, inst. force velocity, inst. movement, static force, and inst. position to inst. inter- spike interval. Comparison between best fit curves suggested that both VP and HP reactivity was best characterized by dynamically applied stimuli (16/19 VP; 10/12 HP). Few units transduced static force or position (4/19 VP and 3/15 HP). VP and HP could not be fit to functions. Conduction velocities were observed to be in the A delta and C fiber range, but due to inaccessibility of many of the receptive fields, could only be obtained in a portion of the population. (Supported by NIDR, DEB0710 and DE7200).

546.2 THE ACTION OF NGF ON SENSORY NEURONS MATURING IN VIVO IS SPECIFIC FOR HIGH THRESHOLD MECHANORECEPTORS (HTMRs). A. M. Ritter and L. M. Mendell

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Dorsal root ganglion (DRG) cells subserve a multitude of physiological functions, and some of this heterogeneity is reflected in somatosensory shape. HTMRs, which convey information about nocuous mechanical stimuli, have long duration, large amplitude spikes with an inflection or "hump" on the falling phase. Low threshold mechanoreceptors (LTMRs) which respond to innocuous stimulation of the skin, have smaller amplitude, shorter duration spikes lacking the hump. NGF is known to be transported by a subpopulation of adult DRG cells (1-3). However, it is not clear if NGF affects HTMRs or LTMRs. We have asked if NGF would affect the development of HTMRs. Animals were divided into SHAM-S and SHAM-T, in which the controls and treated groups respectively, received 2.5 μg of NGF (sonic ag) s.c. from post-natal day (PND) 0-14. When these animals reached adulthood (5 weeks) they were anaesthetized by chloral hydrate. Whole cell recordings were made in dorsal root filaments from Aβ afferents projecting through the SN. NGF treatment did not alter the composition of the adult population (unlike anti-NGF); however, the HTMR population showed a significant (p<0.01) increase in D-2 and HTMRs from adult NGF (1.6-2.9 μg/s.c.) treated animals. Seventy-six percent (28/37) of control HTMRs were slowly-adapting (SA) and 24% (9/37) rapidly-adapting (RA); in contrast, between best fit curves suggested that both VP and HP reactivity was best characterized by dynamically applied stimuli (16/19 VP; 10/12 HP). Few units transduced static force or position (4/19 VP and 3/15 HP). VP and HP could not be fit to functions. Conduction velocities were observed to be in the A delta and C fiber range, but due to inaccessibility of many of the receptive fields, could only be obtained in a portion of the population. (Supported by NIDR, DEB0710 and DE7200).

546.3 REGULATION OF NOCICEPTOR FUNCTION BY NGF IN THE NEONATAL AND ADULT RAT. By G.R. Lewis, A.M. Ritter and L.M. Mendell

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Treatments of rats with anti-sera to NGF has consequences which are specific for nociceptive afferents in the sural nerve (SN) (Ritter et al., Nature 325: 500, 1991). We now ask what are the physiological consequences for these afferents if they are provided with excess NGF in vivo. To examine this question we treated rats with NGF (1-2 μg/s.c.) everyday for the first week then every other day until the end of the treatment. Two treatment regimes were used, neonatal NGF (from birth to 2 weeks) and adult NGF (from 2.5 weeks). When these treatments ceased animals were left to recover for at least 3 weeks, they were then anesthetized with urethane (1.25g/kg) and recordings were made in dorsal root filaments from Aβ afferents (2-17 ms) projecting through the SN. NGF treatment did not alter the make-up of the adult population (unlike anti-NGF); however, the HTMR population showed a significant (p<0.01) increase in D-2 and HTMRs from adult NGF (1.6-2.9 μg/s.c.) treated animals. Seventy-six percent (28/37) of control HTMRs were slowly-adapting (SA) and 24% (9/37) rapidly-adapting (RA); in contrast, between best fit curves suggested that both VP and HP reactivity was best characterized by dynamically applied stimuli (16/19 VP; 10/12 HP). Few units transduced static force or position (4/19 VP and 3/15 HP). VP and HP could not be fit to functions. Conduction velocities were observed to be in the A delta and C fiber range, but due to inaccessibility of many of the receptive fields, could only be obtained in a portion of the population. (Supported by NIDR, DEB0710 and DE7200).

546.4 THE POST-NATAL DEPENDENCE OF MYELINIZED NOCICEPTORS ON NGF IS NOT MEDIATED BY CELL DEATH. By L.M. Mendell, G.R. Lewis and A.M. Ritter

Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY11794

By chronically treating animals with anti-sera to 2.5 μg (anti-NGF treatments) it is possible to produce an almost total depletion of Aβ (2-13 ms) high threshold mechanoreceptors (HTMRs) with a concurrent increase in D-2 and HTMRs (5-16 μg/s.c.) from post-natal day (PND) 0-14 and from PND 2-14. When these animals reached adulthood (5 weeks) they were anaesthetized (urethane); all Aβ DRG cells were readily available for cell counting by Nissl staining method. Several significant (p<0.01) increases were observed. This results in decrease (p<0.01) in D-2 and HTMRs from adult NGF (1.6-2.9 μg/s.c.) treated animals. Seventy-six percent (28/37) of control HTMRs were slowly-adapting (SA) and 24% (9/37) rapidly-adapting (RA); in contrast, between best fit curves suggested that both VP and HP reactivity was best characterized by dynamically applied stimuli (16/19 VP; 10/12 HP). Few units transduced static force or position (4/19 VP and 3/15 HP). VP and HP could not be fit to functions. Conduction velocities were observed to be in the A delta and C fiber range, but due to inaccessibility of many of the receptive fields, could only be obtained in a portion of the population. (Supported by NIDR, DEB0710 and DE7200).


Pac. Med., Memorial Univ. of Nfld., St. John’s, Nfld. Canada A1B 3V6 and Dept. of Pharmacol. and Ther., Univ. of Calgary, Calgary, Al. Canada T2N 4N1

Previous work in our laboratory has suggested the involvement of NO (nitric oxide, a) in non-adrenergic non-cholinergic inhibition at the oesophageal muscular mucous (OMM) of the rat oesophagus. Since diaphragm reportedly is a marker for NO-synthesizing containing neurons in the CNS and PNS, we have examined in our previous study and distribution in this organ. Oesophagi of Sprague-Dawley rats were stretched, dissected into two layers viz, TMM and tunica mucosa externa (TME) and fixed in paraformaldehyde prior to staining. Diaphragm was reacted with NADPH-diaminobenzidine as substrate. In adult animals, stained nerve cells were found throughout the myenteric plexus (MP) as solitary neurons or in groups of up to 5 per ganglion. Stained cells and terminals were present in both TME and TMM. Staining was identical with NADPH or DABH as substrate. In adult animals, stained nerve cells were found throughout the myenteric plexus of either young or old animals. We conclude : 1. Use of DABH as substrate does not reveal a unique neuron population in the oesophageal MP. 2. The histochemical activity observed is probably due to DT-diaphorase. 3. In oesophageal MP neurons this enzyme is probably colocalized with markers other than those implicated in CNS neurons. 4. An association of DT-diaphorase with NO-synthesizing containing neurons cannot be ruled out. (Supported by MRC, Canada.)

Although muscle pain is a common feature in athletics and in various diseases, little is known about the role of muscle nociceptors. We have used intravenous microstimulation and recording in human nerve fascicles supplying skeletal muscles to identify muscle nociceptors. During intravenous stimulation of common peroneal nerve fascicles, seven subjects identified and mapped a field of deep cramp-like pain projected to muscle. Through natural stimulation with mechanical pressure on projected fields, receptive fields of mechanoreceptor units with moderate to high threshold could be located within or adjacent to the areas of projected pain. Conduction velocities ranged 3.1-13.5 m/s for Group III (α-7) and 0.6-1.9 m/s for Group IV (c-α) fibers. None of the Group III or Group IV units were spontaneously active. Mean receptive field areas, mapped with a 3 mm² blunt probe, were 3.2 ±0.84 and 4.7 ±0.03 cm² for Group III and Group IV afferents, respectively. The initial area of projected pain experienced immediately upon intravenous stimulation at threshold for pain sensation ranged from 0.78-20.4 cm².

Continuous intravenous stimulation at steady intensity resulted in gradual enlargement of the painful area. Injection of 0.1% capsaicin into one receptive field caused broad cramp-like pain: the recorded Group III nociceptor became spontaneously active. This constitutes the first electrophysiological characterization of both Group III and IV human muscle nociceptors, and a direct endorsement that their activity evokes cramp-like pain. Supported by NIH NS 24766 and 28747.


Capsaicin evokes an initial neurogenic response that decreases upon repeat stimulation. Plasma extravasation (PE) was used to measure neurogenic response and was determined by measuring the increase in Evans blue dye content of skin from the dorsal hindpaw evoked by topical capsaicin application or antidromic stimulation of the saphenous nerve. Capsaicin (0.15%-1.0%) was applied topically either 1 time/day for 1 day, 1 time/day for 3 days or 3 times/day for 1 week. Plasma extravasation of capsaicin-evoked PE, it failed to affect antidiromically-stimulated PE. To determine whether desensitization represents a specific loss of capsaicin sensitivity or a generalized loss of chemosensitive reactivity, either xylene (100%) or mustard oil (1%) was used to evoke PE. Single application of capsaicin produced a marked reduction in both xylene- and mustard oil-evoked PE. The effect of frequency of capsaicin dosing on block of neurogenic inflammation was also examined. Capsaicin (0.15%-1.0%) was applied topically every 1 hour/day for 1 day, every 3 hours/day for 4 days, or 3 times/day for 1 week. Whereas a single desensitizing application of capsaicin failed to inhibit antidiromically-stimulated PE 24 hours after dosing, capsaicin applied 3 times daily for 1 day did. These effects were dose-dependent. These data indicate that desensitization and block of neurogenic inflammation are distinct and separable actions of capsaicin. Desensitization to capsaicin is accompanied by a generalized loss of chemical sensibility in unmyelinated sensory afferent fibers.

546.7 RUTHENIUM RED ANTAGONISM OF CAPSAICIN'S INHIBITORY EFFECT ON C-FIBERS IN THE ELECTRICALLY-INDUCED PLASMA EXTRAVASATION MODEL. M.M. Roller* and J.N. Meyer. Procter & Gamble Company, Cincinnati, OH 45239-8707.

Ruthenium Red (RR) is an inorganic dye which has been used in studies to antagonize the actions of capsaicin on sensory auditory and their terminals. In these studies the effect of RR on capsaicin inhibition of electrically-induced cutaneous plasma extravasation (P.E.) was investigated. Ruthenium Red or vehicle was injected near the saphenous nerves of male, Sprague-Dawley rats, followed 15 minutes later by capsaicin or vehicle injection. Two hours later the blue dye was injected into the jugular vein and the saphenous nerves were electrically stimulated to produce PE. RR control studies showed no significant difference in P.E. between RR- or saline-treated limbs. In capsaicin control studies, 0.327 mm capsaicin inhibited P.E. by 47.8%, while 3.27 and 16.35 mm capsaicin inhibited P.E. by 72% and 87.8%, respectively. In experimental studies, the inhibitory effect of 0.327 mm capsaicin was antagonized by all concentrations of RR tested, while 3.27 mm capsaicin was antagonized by 10 mM RR, but not by 0.1 mM RR, and 16.35 mm capsaicin was antagonized by 10 mM RR but not by 0.1 or 1.0 mM RR. This dose-responsive effect of RR on capsaicin inhibition which shifts the capsaicin dose-response curve to the right and down suggests that RR may be a competitive antagonist of capsaicin.

546.8 CONDUCTION VELOCITY (CV) OF RAT PRIMARY AFFERENT NEURONES WITH PERIPHERAL-LIKE IMMUNOREACTIVITY (PER-LI) OR CARBONIC ANHYDRASE ACTIVITY (CA-IV) IN THE RAT HINDPAW SKIN. M. Hallabati*, Y. CAWCHE, Y. Barot, M. Hasnain and J. Ochoa. Good Samaritan Hosp. and Med. Ctr., 3795 Main St., Cincinnati, OH 45239.

Rats were used to examine peripheral-like immunoreactivity (PER-LI) and carbonic anhydrase (CA-IV) in the rat hindpaw skin. The methods used were immunohistochemistry and histochemistry. PER-LI and CA-IV were detected in primary sensory afferent neurons. PER-LI and CA-IV were detected in primary sensory afferent neurons. PER-LI and CA-IV were detected in primary sensory afferent neurons. PER-LI and CA-IV were detected in primary sensory afferent neurons.

546.9 AXON REFLEX VASODILATATION IN CAT TOOTH-PULP. B Matthews and N Yongvanit AD. Dept. of Physiology, University of Bristol, Bristol BS8 1TD, England.

In recent experiments we have shown that electrical stimulation of the peripheral cut end of the inferior alveolar nerve to a cat causes both pulpal vasodilatation and an increase in the rate of outward fluid flow through exposed dentine in the lower canine tooth. These effects required the recruitment of small Aδ and C fibres; stimulating the Aβ fibres alone had no effect. In the present experiments we have characterized by their response to local mechanical and chemical mediators of nociception. Conduction velocities ranged 3.1-13.5 m/s for Group III (α7) and 0.6-1.9 m/s for Group IV (c-α) afferents. After recordings were complete, a fluorescent dye (ethidium bromide or Lucifer yellow) was injected into the soma. The DRGs were then fixed with Zamboni's fixative and frozen serial 7μm sections cut. PER-LI was examined using a polychromal antibody, and a generalized loss of chemosensory reactivity.

These data indicate that PER-LI is in all neurofilament (NF) negative and a few NF positive rat dorsal root ganglion (DRG) neurons with all small somata (Fort et al., Brain Res. 515:39-330, 1990). Since NF negative rat DRG neurons have C fibres, it seems possible that PER-LI was in all C- and some or all Aδ neurones. If, as previously proposed (Stabolec et al., Brain Res. 412:29-19, 1987), CA-IV is located on type I and II proprioceptive afferents it should be expressed only in fast conducting Aδ neurones.

We have made microneedling recordings from L3, L4 and L5 DRG in vivo at 36.5°C, taken from postobesed anesthetized 6-8 week female Wistar rats. We have characterized their peripheral nerve CV into Aβ (>12m/sec); Aδ- (1.5-12m/sec) and C-neurones (<1.5m/sec). After recordings were continued, a fluorescent dye (ethylene bromide or Lucifer yellow) was injected into the soma. The DRGs were then fixed with Zamboni's fixative and frozen serial 7μm sections cut. PER-LI was examined using a polychromal antibody, and a generalized loss of chemosensory reactivity.

These data indicate that PER-LI is indeed only in C- or slowly conducting Aδ neurones, while CA is only in neurones with faster conducting Aδ fibres.

ACKNOWLEDGEMENTS: Thanks go to B. Carttushers for technical assistance and for anti-PER antibody to M-M. Porter. This work was supported by the MRC.

Our results prove that the psychophysical response behavior to controlled CO 2 pulses and the respective discharges of MDH neurons are closely related. For noxious stimulation of the nasal mucosa an olfactometer presenting CO 2 pulses and the respective discharges of MDH neurons are closely related. For noxious stimulation of the nasal mucosa an olfactometer presenting

547.1


For noxious stimulation of the nasal mucosa an olfactometer presenting precisely controlled CO 2 pulses was used. These stimuli were embedded in a constant flow of warm and humidified air (flow rate in rat: 6 ml/s; in human: 65 ml/s).

Twelve volunteer subjects rated their pain intensities with a VAS. Randomized CO 2 concentrations ranging from 50-85% (pulse duration 2s) were used to plot the respective stimulus-response functions (SRF). The effect of stimulus duration (CO 2 -pulses ranging from 0.125 - 4s, constant concentration) was examined in addition. Electrophysiological experiments with comparable stimulation protocols were performed in halothane anesthetized rats. The interstimulus interval was 30s in all the tests. In both psychophysical and electrophysiological experiments we found consistent and reliable linear SRFs to different concentrations of CO 2 in humans, pain threshold responses of fibers lasting up to 1s and then reached a plateau level. This finding was paralleled in a subpopulation (11 of 20) of rat MDH neurons which displayed a phasic discharge behaviour to whatever stimulus duration being presented. The other neurons showed phasic and tonic responses.

Our results prove that the psychophysical response behavior to controlled CO 2 pulses and the respective discharges of MDH neurons are closely correlated. The method described here thus provides a precise tool for the quantitative physiological and pharmacological investigation of chemically induced pain. (supported by the DFG)

547.2


Two peripheral nerve manipulations that cause long term effects on nociception are (1) loose ligature (a manipulation that decreases nociceptive thresholds and in a possible chronic pain model) and (2) capsaiscin (a C-fiber neurotoxin that increases nociceptive thresholds) administered. We examined the effects of these manipulations on nerve fiber caliber and on glutamate and SP (two neurochemicals implicated in mediation of nociception) immunoreactivity in the superficial laminae of the dorsal horn of adult rats 2-4 weeks after loosening ligature (2-3 months after applying capsaiscin to the right saphenous nerve. Fiber caliber and density of immunoreactivity were quantified using video microscopy.

Ligature resulted in an 18 to 54% drop in mean cross sectional area of sciatic nerve myelinated fibers on the ligature side relative to the control side (previous studies have shown that capsaiscin impairs unmyelinated fibers). Furthermore, ligature reduced SP immunoreactivity by 18 to 36% compared to the control side but it did not affect the density of glutamate immunoreactivity. Conversely, capsaiscin application did not affect the density of SP immunoreactivity but it resulted in an increase of 10% in the density of glutamate immunoreactivity as compared to control. Thus, differences in the effects of loose ligature and capsaiscin on behavior are paralleled by differences in their effects on nerve structure and on immunoreactivity in the dorsal horn. Additional work is underway to examine the effects of the two manipulations on NMDA receptors in the superficial dorsal horn.

547.3


Afferents from the cervix arrive in the rat spinal cord in two distinct areas, S1 and L1. Sixty-three cells which responded to pressure on the cervix were examined in these two areas in 7 nulliparous rats in estrus. The rats were decerebrated and had a TiO injection under brief anesthesia. In addition to the cervix, mechanical stimulation was applied to the uterus, colon and skin. Only minimal stimuli were used to avoid long-term sensitization. In the S1 segment, 33 cervix-responding cells were found in all dorsal horn laminae. All these cells had extensive cutaneous receptive fields and 45% also responded to the colon (39% excited, 6% inhibited). In addition, 42% responded to the uterus (18% inhibited by one uterine horn and excited by the other, 18% inhibited by the contralateral or both horns, 6% excited by either horn). In the L1 segment, 30 cervix-responding cells were found in the L1 segment. All these cells had extensive cutaneous receptive fields and 57% were excited by the colon, while 76% were excited and 8% inhibited by uterine distension of either horn. Thus, in both areas, all cervix-responding cells had large cutaneous receptive fields but they were differentiated by their location and by the degree of convergence from other pelvic structures. (Supported by NIH grant ROI NS11892.)

547.4


Experiments were conducted to determine the effect of tonically active descending pathways on thoracolumbar spinal neurons that respond to renal nerve stimulation in anesthetized cats. The influences of tonic descending controls on seventy-two spinal neurons were assessed by examining changes in cell discharge rate associated with reversible block of spinal cord conduction by local cooling. We determined the effect of spinal cold block on spontaneous activity, neuronal responses evoked by renal afferent nerve stimulation and neuronal responses to somatic stimulation. The interruption of descending pathways by cold resulted in enhanced spontaneous (tonically inhibited neuronal), reduced (tonically excited neuronal), or unchanged neuronal responses. The spontaneous activity of 61% of the neurons increased from 7.4 ± 2.1 imp/s before cooling to 26.1 ± 5.0 imp/s during cooling. A decrease in activity was observed in 10% of the neurons and 29% of the neurons had no change in activity. Cooling increased 75% of cell responses to renal nerve stimulation. Approximately a two fold increase in the spikes/stim for both A-delta and C-fiber mediated responses was observed. In addition, four neurons had C-fiber input which was revealed by cold block. Decreased responses to A-delta and C-fiber input occurred in 13% of the neurons during cooling. Twelve percent of the cell responses to renal nerve stimulation were not affected by cold block. All but one of the unchanged responses were mediated by A-delta input. All neurons had somatic receptive fields and most exhibited increased responses to somatic stimulation during cooling. Thus, tonic descending controls increased cell responses to renal nerve stimulation, increased cell responses to somatic stimulation and therefore were predominantly modulated by tonic descending inhibitory influences. Supported by NIH Grant HL36367.
547.5 MATURATION OF APV- AND SPANTIDE-SENSITIVE SLOW VENTRAL ROOT POTENTIALS IN RAT SPINAL CORD. L. M. Gibbs, J. J. Kendig. Dept. of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5123

The neonatal (1-6 day old) rat spinal cord exhibits a slow ventral root potential (slow VRP) with a long duration (10-40 s). Dorsal root-evoked motor neuron responses in older animals are reported to be of much shorter duration (1-3 s). We have investigated the maturation of the slow VRP in isolated superfused (27-28°C) spinal cord preparations from rats of ages 1-21 days, intact, heparinized or prepared as thick transverse slices. In cords from animals 1-9 days old, a single stimulus to a lumbar dorsal root evoked a monosynaptic reflex followed by a slow VRP whose corresponding ipsilateral ventral root; maximum amplitude of the slow VRP was at 3-4 s. Both the substance P antagonist spantide (10 µM) and the NMDA receptor antagonist APV (20 µM) depressed the slow VRP. Cords from 12-21 day animals displayed a VRP of 3-4 s duration, with maximum amplitude at 3 s after the stimulus. APV, but not spantide, depressed this response; low Mg+2 increased the amplitude but not the decay constant. These results suggest that a very slow substance P-mediated component of the VRP is selectively lost or inhibited at 10-11 days postnatal development in rat spinal cord.

547.6 TONIC DESCENDING INHIBITION OF SLOW VENTRAL ROOT POTENTIALS IN ISOLATED NEONATAL RAT BRAINSTEM-SPIRAL CORD. A. Tarasiuk*, J. Blundell, A. Demuro, D. A. Bredt, J. S. Arimura, M. J. Parnas, J. P. Lipton, Dept. of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123

Descending inhibition from brainstem nuclei modulates responses to noxious stimuli; its presence in the neonatal rat is controversial. We examined the extent of tonic descending inhibition in brainstem-spinal cords isolated from 1-6 day old Sprague-Dawley rat pups. Brainstem and lumbar regions were superfused separately. Slow ventral root potentials (slow VRP) were recorded at the corresponding ipsilateral ventral root. Integrity of brainstem-spinal cord connections was verified by evoking a mono-synaptic reflex from the trigeminal nerve root to the lumbar ventral root. Three different manipulations were used to assess descending modulation: severing the brainstem from the cord, cooling the brainstem from 28 to 15°C, and perfusing the brainstem with the local anesthetic lidocaine (1-5 µM). In each case the amplitude of the lumbar slow VRP increased, by as much as 25%. The effects of cooling and of lidocaine were reversible. Descending projections from brainstem nuclei are anatomically present in the neonate; these results show that they functionally inhibit slow spinal reflexes.

547.7 HYBRIDIZATION HISTOCHEMICAL ANALYSIS OF SPINAL NEURONS THAT EXPRESS THE a2 ADRENERGIC RECEPTOR IN A RAT MODEL OF PERIPHERAL MONONEUROPATHY. Frank Williams, Angela Bimbbaum, George Wilson, and Alvis Beitz. Department of Veterinary Biology and Pharmacology, University of Minnesota, St. Paul, MN 55108

Several recent reports have implicated brainstem-nordnergic projections to the spinal cord in the modulation of acupuncture. The present studies sought to determine whether chronic pain is associated with a physiologic response to increased adrenergic activity in the spinal cord dorsal horn. Specifically, we sought to test whether the biosynthesis of a2 adrenergic receptor mRNA is altered. Uterine acyclic ligaments were placed in male Sprague-Dawley rats according to the method of Bennett and Xie (Pain 33:87). Hyperalgesia was confirmed by increased licking and pinching of paw withdrawal following the plantar application of radiant heat. Difference scores averaged 6.2% greater on the ligatured side than on the unligatured side (P<0.001). The ligatures were removed under Nembutal anesthesia, cryosectioned (15μm), and the sections prepared for hybridization histochemistry. The sequence of the oligo-DNA probes (48mer) were complementary to unique portions of the mRNA encoding the large cytoplasmic loop of the human a2-C10 or the a2-C4 subtypes. Control probes either matched the mRNA or had random sequences. The hybridization patterns in the dorsal horn on the ligatured side differed from the control side by two features. First, in the lateral portion of lamina I, significant increases in the a2 mRNA hybridization signal were noted on the ligatured side. Second, on the individual probe, calibrated gray levels from video micrographs were 111% to 120% of those on the unligatured side, while background gray levels lowered to an average of 64% of the unligatured side (P<0.05, ANOVA & Fisher PLSD). Hybridization-positive neurons were largely found in lamina II. The results suggest that a chronic pain associated is with increases in a2 adrenergic receptor mRNA.

547.8 DESCENDING FACILITATION AND INHIBITION OF SPINAL MECHANICAL TRANSMISSION. M. Zhuo and G.F. Gebhart. Dept. Pharmacology, Univ. Iowa, Iowa City, Iowa 52242.

The present work investigated descending modulation from nuclei reticularis gigantocellularis and gigantocellularis pars alpha (NGC/NGCa) spinal (noxious pressure and non-noxious brush) transmission of class 2 (WDR) cells. Electrical stimulation at 26/46 sites in the NGC/NGCa produced, for spinal, sensory (n-) or only inflammatory (n+) responses to non-nociceptive mechanical pressure. Electrical stimulation produced biphasic (n+), only facilitation (n+), or only inhibition (n+). Unit responses showed facilitation of responses to noxious mechanical pressure. Local anesthetics of the vonalventral funiculi (VLF) selectively abolished descending facilitation and dorsal funiculi (OLF) selectively abolished inhibition. These results suggest that: (1) descending facilitation modulation from the NGC/NGCa is qualitatively the same on nonspecific and nociceptive mechanical; (2) descending facilitation and inhibition are conveyed in the VLFs and OLs, respectively.

547.9 LIDOCAINE INHIBITION OF HYPERACTIVE DORSAL HORN NEURONS IS NOT MEDIATED BY SUPRASPINAL SYSTEMS. P. Marchetti*, M. Lacerenza*, M. Sotgiu *1, S. Smirne*, F. Lacquaniti. Istituto Scientifico H San Raffaele Depts. of Neuroanatomy and C.N.R. *1 Milano, Italy.

Previous studies have shown an inhibitory action of systemic lidocaine on differentially injured dorsal horn neurons. To further the mechanism of this action a model of neuropathic pain was prepared in 20 adult male Wistar rats. All animals received 4 ligatures of the sciatic nerve of one limb. Signs of pain behavior were checked daily. Acute experiments were performed in male Sprague-Dawley rats according the the method of Bennett and Xie (Pain 33:87). Hyperalgesia on the ligatured side was confirmed by comparing the latency of the slow VRP which averaged 5.2 sec. Sixteen to 18 days following ligation, lumbar spinal cords were removed under Nembutal anesthesia, cryosectioned (15μm), and the sections prepared for hybridization histochemistry. The sequence of the oligo-DNA probes (48mer) were complementary to unique portions of the mRNA encoding the large cytoplasmic loop of the human a2-C10 or the a2-C4 subtypes. Control probes either matched the mRNA or had random sequences. The hybridization patterns in the dorsal horn on the ligatured side differed from the control side in two ways. First, in the lateral portion of lamina I, significant increases in the a2 mRNA hybridization signal were noted on the ligatured side. Second, on the individual probe, calibrated gray levels from video micrographs were 111% to 120% of those on the unligatured side, while background gray levels lowered to an average of 64% of the unligatured side (P<0.05, ANOVA & Fisher PLSD). Hybridization-positive neurons were largely found in lamina II. The data suggest that chronic pain is associated with increases in a2 adrenergic receptor mRNA.


The present work investigated descending modulation from nuclei reticularis gigantocellularis and gigantocellularis pars alpha (NGC/NGCa) spinal (noxious pressure and non-noxious brush) transmission of class 2 (WDR) cells. Electrical stimulation at 26/46 sites in the NGC/NGCa produced, for spinal, sensory (n-) or only inflammatory (n+) responses to non-nociceptive mechanical pressure. Electrical stimulation produced biphasic (n+), only facilitation (n+), or only inhibition (n+). Unit responses showed facilitation of responses to noxious mechanical pressure. Local anesthetics of the ventrolateral funiculi (VLF) selectively abolished descending facilitation and dorsal funiculi (OLF) selectively abolished inhibition. These results suggest that: (1) descending facilitation modulation from the NGC/NGCa is qualitatively the same on nonspecific and nociceptive mechanical; (2) descending facilitation and inhibition are conveyed in the VLFs and OLs, respectively.

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THURSDAY PM
547.11 Electroacupuncture modifies the expression of c-fos in the spinal cord induced by noxious stimulation. J. H. Lee, D. R. Brown and A. A. Becker. Dept. of Biophysics, Univ. of Texas, M. D. Anderson Cancer Center, Houston, TX 77030. Electroacupuncture is thought to be mediated in part by descending pain modulation pathways that impinge on nociceptive neurons in the spinal cord dorsal horn. Many investigators have suggested that these descending pain control pathways (DCPs) are mediated by endogenous opioid mechanisms for their analgesic effect, whereas non-opioid systems have important roles for the analgesic effect of high frequency electro-acupuncture (EA). In the present study was done to investigate the effect of EA on c-fos expression in the spinal cord induced by noxious stimulation and to evaluate the opiate sensitivity of 4 Hz versus 100 Hz EA applied bilaterally for 60 min duration to the point Zusanli (S36) located in the hindlimb. Mechanical NS (10 sec/min for 10 min) was applied to the right hindpaw following 30 min of EA treatment and the resulting c-fos expression in the spinal cord dorsal horn was compared to that obtained in rats only exposed to the noxious stimulation. The opiate sensitivity of the 4 Hz versus 100 Hz stimulation frequency was evaluated by giving naloxone (0.5mg/kg) treatment 10 minutes before initiating EA. Both 4Hz and 100Hz EA reduced the number of c-fos-immunoreactive neurons in the dorsal horn induced by noxious stimulation by 58% and 50%, respectively. The suppression of c-fos expression induced by 4 Hz EA was completely reversed by prior treatment with naloxone. On the other hand, the suppression of c-fos induced by 100Hz EA was only partially blocked by this opiate antagonist. These data indicate that both high and low frequency EA inhibit the expression of c-fos in the dorsal horn induced by noxious stimulation and further suggest that low frequency (4Hz) EA is primarily mediated by endogenous opioid systems, while both opioid and non-opioid mechanisms appear to be involved in mediating the analgesic effect of 100Hz EA. Supported by NIH grants DA 06687, DE 06682 and DC 01086.

547.12 Evaluation of the Release of I-CGRP in Dorsal Horn Following Carrageenan Induced Inflammation. Mary G. Garry and Kenneth M. Hargreaves. Univ. of Minnesota, Dept. of Restorative Dentistry, Minneapolis, MN. Superfusion of spinal cord slices is useful for evaluating the regulation of neuropeptide release from central terminals of primary afferent fibers. EA, ketorolac, and glucocorticoids are known to modify the information on the regulation of release from terminals located in peripheral tissue. Bovine dental pulp was selected for these experiments since the tissue is readily available and has a rich supply of primary afferent neurons which are known to contain c-fos immunoreactive GRFP. Mandibular incisors were excised at the slaughterhouse and transported on ice to the laboratory. Pulps were harvested, sliced and chopped (300 μm cubes) with a McIlwain tissue chopper. Tissue (200-250 mg) was superfused with Krebs buffer (400 μM/min, 3 min fractions) for a 120 min baseline recovery period; samples were collected in 100 μl glacial acetic acid and lyophilized. Potassium (60 mM)-evoked release of immunoreactive c-cGRP was measured by a RIA (antiserum kindly provided by Dr. M. L. Harper, Lilly, Indianapolis, IN). Initial studies demonstrated that bovine I-CGRP diluted in parallel to rat I-CGRP in the RIA and was present in dental pulp at a concentration of less than 10% SP depletion, Tau remained at baseline levels throughout the observation period. Stimulation of dental pulp with 60 mM potassium increased superfusate levels (p<0.01) of I-CGRP to 155±21 fmol/G/min (n=7). These studies indicate that superfusion of dental pulp permits evaluation of the regulation of neuropeptide release from peripheral tissue stores.

547.13 TIME COURSE OF AMINO ACID RELEASE INTO THE LUMBAR DORSAL HORN OF THE PRIMATE DURING DEVELOPMENT OF AN ACUTE ARTHRITIS. L. L. Sorkin, K. N. Westlund, K. A. Sluka, and W. D. Willis. Marine Biomedical Institute, Univ. of Texas Med. Branch, Galveston, TX 77550. Extracellular levels of several amino acids were measured in the dorsal horn of anesthetized primates. Half hour continuous samples were obtained via a microdialysis probe before, during and for 4.5 hours after injection of kaolin/carrageenan into the articular capsule of one knee and assayed using an RIA. The release of glutamate, aspartate, glycine and serine increased ipsilaterally to the inflammation following injection of irritants into the knee joint. Within two and control half hours after the injection, these amino acid levels returned to near baseline. Movement of the knee after, but not before inflammation lead to release of these same amino acids. In animals that were found to have depletion of immunoreactive substance P (SP) in the dorsal horn on the side of the inflammation, tauina (Tau) levels rose starting two to three hours after the injection. In animals with less than 10% SP depletion, Tau remained at basal levels throughout the experiment. Thus, during the first stages of joint inflammation, EAAs are released into the dorsal horn. This is followed by increased levels of IAAs, presumably representing activation of descending endogenous analgesia systems. This phase is followed by a somatotopically restricted increase in extracellular levels of SP as well as Tau. While SP is presumably part of a nociceptive transmission system, Tau via its ability to reduce Ca++ influx, could be part of a second analgesia mechanism aimed at semicative and/or chronic pain. Supported by N51355, NS07943 and Bristol-Myers Squibb.

547.14 ADRENALECTOMY ENHANCES C-FOS-LIKE IMMUNOREACTIVITY IN TRIGEMINAL SUBNUCLEUS CAUDALIS IN RESPONSE TO NOXIOUS CORNEAL HEAT STIMULATION. L. L. Sorkin and D. A. Lattier. Section of Neurology & Dept of Surgery, Brown Univ/RH Hospital, Providence, RI 02903. The role of glucocorticoids in somatic sensory function is not well defined, however, receptors for glucocorticoid agonists of the spinal cord and certain forms of stress-induced analgesia may involve adrenal steroids. Expression of the proto-oncogene, c-fos, by nociceptive sensory stimuli induces fos-like immunoreactivity (FLI) in spinal laminae consistent with the location of nociceptor specific neurons. To assess the influence of glucocorticoids on FLI evoked by a well defined nociceptive stimulus, the distribution of FLI-positive neurons within trigeminal subnucleus caudalis (Vc) was quantified in barbiturate-anesthetized rats in response to corneal stimulation of the nasal surface. Noxious (55°C) or innocuous (42°C) thermal stimuli were applied in 30 s pulses for 15 min to adrenalectomized (ADX), adrenalectomized rats given corticosterone replacement (ADX+B), rats were perfused and sections were collected 40 min after corneal stimulation. FLI was quantified by point counting in the superficial laminae of the caudal Vc (460±160 cells). In ADX rats, noxious corneal heat evoked a reliable increase in the number of FLI-positive neurons (255±22 cells) in Vc, whereas in ADX+B rats the number of FLI-positive cells (827±75) was reduced compared to that of ADX rats. Sparse FLI was seen also in Vc at low levels bilaterally, in the deep lamina of ipsilateral caudal Vc and in contralateral caudal Vc in all groups. ADX alone or innocuous corneal heat had little effect on FLI in Vc. FLI was seen also in NTS and ventrobasal thalamic nuclei bilaterally after noxious corneal heat and was increased in ADX compared to intact rats. The results are consistent with the hypothesis that glucocorticoids modify the function of superficial mediolateral dorsal horn neurons in response to noxious trigeminal input. Supported by NIH grant NS26317.

547.15 CHANGE IN GLUTAMATE IMMUNOREACTIVITY IN THE MEDIAL ARTICULARafferent nerve fibers. H. H. Westlund, J. F. Sung, M. P. Dougherty, I. S. Sorkin and T. D. Willis. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550. Glutamate (GLU) and other excitatory amino acids have been shown to play a key role in nociception and the hyperalgesia associated with the acute inflammatory response. In an effort to more fully understand the role of GLU in this process, the percentage of afferent fibers immunoreactive for GLU was determined in the medial articular nerve, a source of perineuralafferent fibers innervating the knee joint of the monkey. After induction of the experimental knee joint inflammation with a kaolin/carrageenan mixture, comparison was made of the percentage of GLU positive axons from the control side versus the side of the inflammation. A doubling in the percentage of GLU axons was observed on the side of the experimental arthritis as compared to the medial articular nerve of the other side suggesting that GLU content is greatly increased in the perineural afferents. These increases were observed only when injection of kaolin/carrageenan was combined with joint flexion and mechanical stimulation in the anesthetized preparation. GLU positive axons were of small diameter and unmyelinated or were included in the thinly myelinated group in the control nerve. Following induction of the inflammation, axonal diameter determination revealed an increase in the percentage of thinly myelinated axons which would correspond to the A delta fiber type. An increase in GLU content could explain the enhanced mechanosensitivity and increased reactivity observed in afferents in this model of inflammation. These findings implicate increased GLU as a causal factor in the lacinating pain of movement and hyperalgesia experienced in the inflammatory state. (Supported by grant #NS11255, NS07943, NS08686, RDCA NS01445-02 and Bristol-Myers Squibb.)
547.17

SUBSTANCE P, CALCITONIN GENE-RELATED PEPTIDE AND GLUTAMATE ALTERATIONS IN THE SPINAL CORD OF THE PRIMATE DURING ACUTE ARTHRITIS. K.A. Shukla, P.M. Dougherty, L.S. Sokrin, W.D. Willis and K.N. Westland. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The effects of an experimentally induced arthritis on immunoreactivity of putative primary afferent neurotransmitter/neuromodulators were examined. Immunoreactive staining for substance P (SP), calcitonin gene-related peptide (CGRP), and glutamate immunoreactivity (Glu) in the monkey lumbar spinal cord were examined following inflammation of one knee joint induced by injection of kaolin and carrageenan (5% and 5%). Spinal cords were examined at different time periods after induction of arthritis (2.5, 4, 6, and 8 hours). Side-to-side differences in the density of immunoreactive staining were determined by a computer-assisted quantitation system. A significant decrease in immunoreactivity of the ipsilateral dorsal horn was found for SP at 4, 6, and 8 hours. The decrease for SP was 68.3% at 4 hours, 54.7% at 6 hours, and 65.5% at 8 hours. Immunoreactivity for CGRP was significantly decreased 31.5% after 8 hours. Immunoreactivity for Glu, on the other hand, showed an increase ipsilaterally of 31.4% at 4 hours, 33.7% at 6 hours, 39.9% at 8 hours. The frequency of peripheral stimulation of the joint was shown to be important for changes in Glu and SP immunoreactivity. Without frequent stimulation joint in the early stages of the development of arthritis, increases in Glu immunoreactivity were not as prominent. In these same animals SP and CGRP showed no quantitative side to side differences without the joint stimulation. These studies suggest that GLU may be involved in arthritic pain at rest, whereas SP, CGRP and GLU may mediate pain induced by joint movement.

(Supported by grants NS11253, NS09743, NS08860, R0CA NI01445 and Bristol- Myers Squibb).

547.19

POTENTIAL OF RESPONSES OF MONKEY SPINOThALAMIC (STT) CELLS TO EXCITATORY AMINO ACIDS ACCOMPANIES THE DEVELOPMENT OF Capsaicin-INDUCED HYPERALGESIA. W.D. Willis and P.M. Dougherty. Marine Biomedical Institute, 200 University Blvd, UTMB, Galveston, Texas 77550-2772.

The present study addresses the role that excitatory amino acids (EAA's) may play in the sensitization of STT neurons by intradermal (i.d.) capsaicin (CAP). A total of 10 young adult monkeys (Macaca fascicularis) were anesthetized and a laminectomy performed to expose the lumbar enlargement. STT neurons located in segments L4-L6 were recorded with carbon filament containing multibarrel electrodes. STT cells were stimulated and identified by use of antidromic potential (AP) or through electrode placed within the ventral posterior lateral nucleus of the thalamus. The responses of the STT cells to mechanical stimulation of the receptive fields and to chemical stimulation by iontophoretically released glutamic, aspartic, quisqualic and N-methyl-D-aspartate (NMDA) acids were recorded before and following injection of capsaicin. Capsaicin was injected at a concentration of 3% in 0.1 ml of a mixture of ethanol/tween 80 and saline into the field of each cell. Testing of responses to mechanical and chemical stimulation began at 30 minutes after injection and was continued for up to two hours afterward. Our results show that the responses of STT cells to N-methyl-D-aspartate, glutamate, aspartate, and quisqualate became increased in those cells which also showed increased mechanical responses following i.d. CAP. Finally, in each of the cells showing an increase in response to mechanical stimulation, the responses to at least one EAA (and often more than one EAA) became increased with a time course identical to that for the mechanical responses. (Supported by NS08860, NS09743 and NS11253).

547.18

ENHANCEMENT OF RESPONSES OF MONKEY SPINOthalamic CELLS TO EXCITATORY AMINO ACIDS ACCOMPANIES THE DEVELOPMENT OF ACUTE ARTHRITIS. P.M. Dougherty, K.A. Shukla, L.S. Sokrin, K.N. Westland and W.D. Willis. Marine Biomedical Institute, 200 University Blvd, UTMB, Galveston, Texas 77550-2772.

The experiments described here show a potentiation of the responses of spinothalamic tract (STT) neurons to both chemical and mechanical stimuli in monkeys during the development of an acute arthritis. A total of 12 young adult monkeys (Macaca fascicularis) were anesthetized and a laminectomy was performed to expose the lumbar enlargement. STT neurons in the L4-L6 segments were recorded with carbon filament containing multibarrel electrodes. STT cells were isolated and identified by use of antidromic potentials passed through an electrode placed in the ventral posterior lateral nucleus of the thalamus. The responses of STT cells to mechanical stimulation of the receptive fields and to chemical stimulation by iontophoretically released glutamic, aspartic, quisqualic, and N-methyl-D-aspartate acids were recorded before and following induction of arthritis. Arthritis was produced by injection of 0.5 ml of a 5% kaolin-5% carrageenan mixture into the knee joint capsule. The results of this study show an increase of the responses of 8 of 12 cells to non-nociceptive flexion of the knee joint following this injection. The responses of these cells to nociceptive pinch of the skin were also enhanced. The enhancement of both these responses followed a similar time course, with onset of changes one to two hours following injection of the knee joint. The results also demonstrate that the increase in responses of STT neurons to mechanical stimuli is accompanied by an increase in response to iontophoretically applied excitatory amino acids (EAA's), particularly those acting at non-N-methyl-D-aspartate (non-NMDA) receptors (e.g., glutamate, aspartate and quisqualate). (Supported by NS08860, NS09743 and NS11255).

547.20

NEUROPLASTIC EFFECTS INDUCED BY LINGUAL INFLAMMATORY IRRITANT ON CUTANEOUS AND DEEP RECEPTIVE FIELD PROPERTIES OF NEURONS IN TRIGEMINAL (V) SUBNUCLEUS CAUDALIS. X.-M. Xu, J.W. Hu and R.J. Sostek. Faculty of Dentistry, Univ. of Toronto, Ont. M5G 1G6 Canada.

We have documented that the inflammatory irritant mustard oil injected into the masseter muscle can change the cutaneous receptive field (RF) properties of caudalis nociceptive neurons. This study's aim was to characterize the properties of caudalis neurons receiving deep as well as cutaneous RF inputs and to test the effect on them of mustard oil. Of 196 single neurons recorded in V subnucleus caudalis in 15 anesthetized rats, 74% received cutaneous input only, 14% deep input only and 12% were neurons (CD) receiving nociceptive cutaneous and deep inputs. Reversible effects of mustard oil (5μl) injected into the tongue on cutaneous and deep RFs were observed in 8 of 9 CD neurons tested. There was a significant increase of both cutaneous and deep RFs, and, compared with the effect of vehicle injection, the expansion of deep RFs was significantly greater (45% p < 0.01) and longer in duration (10-15 min); also, deep RFs expanded more than cutaneous RFs (p < 0.05). These results provide further support for injury-induced neuroplasticity in V nociceptive neurons and suggest that deep inputs may particularly manifest this effect. Supported by NIH grants DE04786 and DE05559.

PAIN MODULATION: SPINAL AND TRIGEMINAL THURSDAY PM

548.1


Capsazepine depolarizes and excites a subset of primary afferent, sensory neurons by opening a membrane ion channel that is permeable to monovalent and divalent cations. This action is shared by resiniferatoxin (RFTX). In ion flux studies, capsazepine antagonized the capsaicin induced excitation of neonatal rats. Capsazepine (0.5-1 μM) reversibly reduced or abolished the increase in response to mechanical stimulation the responses to at least one mechanical responses following i.d. CAP. Finally, in each of the cells showing an increase in response to mechanical stimulation, the responses to at least one EAA (and often more than one EAA) became increased with a time course identical to that for the mechanical responses. (Supported by NS08860, NS09743 and NS11253).

548.2


There are several lines of evidence indicating that the selective excitatory action of capsaicin on primary sensory neurons is produced by activation of a specific receptor (Bever and Szolcsanyi, 1990). The capsaicin receptor has been recently supported by using a competitive capsaicin antagonist, capsaazepine (Bever et al., 1991). We now report a selective antagonism of the capsaicin-induced activation of C-fibres, using two in vitro preparations: the mouse spinal cord - DRG, and the rat hindpaw skin - saphenous nerve. Capsazepine (0.2-1.0 μM, 30 sec) applied to the receptive field of single primary afferent fibers selectively excited C- and A-delta mechan-heat receptors. Capsazepine (0.01-0.5 μM, 5 min) perfused to the receptive field prior to capsaicin significantly reduced the firing evoked in single fibers. In the spinal cord a majority of dorsal horn cells were depolarised when primary afferents were activated by capsazepine (0.04-0.7 μM) applied to the DRG. Superfusion of the DRG (but not the spinal cord) with capsazepine blocked the capsaicin-induced dorsal horn depolarization. Depolarization evoked in the same cells by application of substance P (0.5 μM) to the cord was unaffected by capsazepine. The antinociceptive effects of capsaazepine were reversible, and similar concentrations were ineffective when administered alone in both preparations. These data show that a novel capsazepine receptor exists in sensory neurons and support the concept that capsazepine activates C-fibers via a specific receptor site.


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548.3

CAPSAICIN ANTAGONISED BY CAPSAZEPINE: IN VIVO STUDIES. M.N.Perkins, A.Dray, and E.A.Campbell.* Sandoz Institute for Medical Research, Luton, Bedfordshire, UK.

Capsaicin has been shown to be a selective, competitive antagonist of capsaicin-induced activation of sensory neurones in vitro (Bevan et al, 1991) and in vivo (Dray et al, 1991). We show that capsaicin antagonises both capsaicin-induced agonist responses and antinociceptive effects in vivo. The capsaicin-evoked reflex blood pressure fall in anaesthetised rats was blocked by the bromochonosteroid CQ in acute, but not after repetitive nerve stimulation in anaesthetised guinea pigs were both antagonised by capsaicin infused at 0.2-0.5 μmol/min for 5 min. In carageenin-induced paw pressure hyperalgesia in rats the antinociceptive action of capsaicin (20μmol/kg sc) was reversed by 100μmol/kg sc capsaicin. In the tail-flick model in rats and mice capsaicin (100μmol/kg sc) prevented the antinociceptive action of capsaicin (20μmol/kg sc). Lower doses of capsaicine were generally ineffective. In a model of persistent hyperalgesia, produced by the injection of uric acid into a rat knee joint, the antinociceptive action of capsaicin (20μmol/kg sc) was partially reversed by capsaicin at 100μmol/kg and completely reversed at 200μmol/kg. In each of these models, systemic capsaicine alone was without effect. These data show that capsaicine acts as a capsaicin antagonist in vivo and supports the concept that both the agonistic and antinociceptive actions of capsaicine are mediated by the same specific receptor.


548.4

CAPSAICINE; CAPSAICIN ANTAGONISM AND EVIDENCE FOR A SPINAL MECHANISM IN CAPSAICIN-INDUCED ANTINOCICEPTION. A. Dyer and A. Doidson* School of Pharmacy, London, UK.

The effects of capsaicin are likely to be mediated by a specific receptor. This is supported by recent studies using the competitive antagonist capsazepine (Bevan et al, 1991, Br. J. Pharmacol. 101, 77P). Capsazepine at 100 μmol/kg reduced tail-flick latencies recorded in vitro from the neonatal rat cord preparation following brief application of capsaicin and other nociceptive stimuli. Responses evoked by capsaicin administered to the tail or spinal cord were selectively and reversibly reduced by capsaicine (IC50= 200μM). In anesthetised adult rats, C- but not A-fiber evoked changes of WDR dorsal horn neurones, were reversibly reduced by a low dose of capsaicin (30 μmol/kg s.c.). This was antagonised by capsaicine administered either s.c. (100μmol/kg) or directly to the spinal cord (5mmol in 50μl). Capsaicine alone did not affect neuronal responses to peripheral stimuli. Local injection of capsaicine into the peripheral receptive field first activated C fibres and then reduced their electrical excitability. Co-injection of capsaicine antagonised both these effects of capsaicine. These results show that capsaicine antagonises a number of actions of capsaicine. Specificity of these data support the hypothesis that antinociception, following systemic capsaicine, is due to an effect via capsaicine receptors on spinal afferent fibres.

548.5

EFFECTS OF INTRATHecal CAPSAICIN AND STRYCHNINE AND TOPICAL COLCHICINE ON THE THERMAL HYPERALGESIA FOLLOWING SCIATIC NERVE CONSTRICTION IN RATS. T. YAMAMOTO* and T-L. YAKSH. Depart. Anesth. UCSD, La Jolla, CA 92093.

SCIATIC NERVE CONSTRICTION MODEL IN RATS. T. YAMAMOTO* and K. HARBOR. Dept. of Anesth. UCSD, La Jolla, CA 92093.

A chronic sciatic nerve constriction injury produces ipsilateral hyperalgesia in rats and this hyperalgesia was proposed as an animal model of peripheral neuropathy. This syndrome is thought to reflect a reduction in small fiber afferents consequent to the injury. To investigate possible mechanisms leading to the hyperalgesia, we examined the effects of intrathecal (IT) capsaicin (CAP; C-fiber neuronophagia), produced by the injection of uric acid into a rat knee joint (the antinociceptive action of capsaicin (20μmol/kg sc) was partially reversed by capsaicin at 100μmol/kg and completely reversed at 200μmol/kg). In each of these models, systemic capsaicine alone was without effect. These data show that capsaicine acts as a capsaicin antagonist in vivo and supports the concept that both the agonistic and antinociceptive actions of capsaicine are mediated by the same specific receptor.

548.6


When injected into peripheral tissues or directly onto the spinal cord, prostaglandins (PGs) lower the algesic threshold in animal models of nociception. One possible mechanism to explain this hyperalgesia is that PGs increase the release of neurotransmitters from nociceptive sensory neurons. To test this hypothesis, we studied the effects of PGs on the release of substance P (SP) from rat sensory neurones grown in culture.

Neurons were dissociated from dorsal root ganglia of 16-18 day old rat embryos and grown in culture for 14 days. Release studies were performed by exposing neuronal cultures to Krebs buffer containing 3.5M KCl (basal release) or by removing the 1μM capsaicin, 30M KCl and/or PGs (stimulated release). Buffer was then assayed for SP using RIA.

Exposure of neurons to 1μM capsaicin or 30M KCl resulted in a 3.4 fold increase in SP release in a buffer that was calcium dependent. Addition of 10μM PG2 to the cells significantly increased SP release by 2 fold from 26.9 ± 1.5 pg/well/10 min to 48.5 ± 1.6 pg/well/10 min. In contrast, neither 10μM PGD2 nor 10μM PGE2 produced a significant increase in SP release. PGE2 did, however, enhance the potassium-stimulated release of SP. These results demonstrate that PGs can stimulate release of neurotransmitter from rat sensory neurons and suggest that this effect may contribute to PG-induced hyperalgesia. (Supported by the Arthritis Foundation and NS21697).

548.7

SYSTEMIC MORPINE COMPLETELY BLOCKS ACUTE C-FIBER STIMULATION-INDUCED DEPRESSION OF COPROP AND REDUCES DEPRESSION OF GALANIN. C.M. Klein, C.M. Pover, R.E. Cogentahall, and L.S. Sotkin. The Mariske Biomedical Institute, Dep. of Anatomy and Neurosciences and Dept. of Physiology & Biophysics, The University of Texas Medical Branch, Galveston, Texas 77550.

In past studies, we observed decreases in immunoreactive staining of calcitonin gene-related peptide (CGRP) and galanin (GAL) in L4-L5 dorsal horn following acute stimulation of the sciatic nerve at C- but not A-fiber fiber volleys were monitored from the contralateral side. CAP but not STR or COL treatment resulted in a significant increase in the latency of the normal paw. CAP VEH STR saline COL5 COL50 saline

These results suggested that 1) C-fiber afferents did not play an important role in producing hyperalgesia; 2) glycine receptor activity inhibits developing hyperalgesia.

Hyperalgesia due to peripheral afferent activation.

548.8

SUBSTANCE P (1-7) SPINAL ANTINOCICEPTION AND INTERACTION WITH THE β-FNA-SENSITIVE OPIATE RECEPTOR SUBTYPE. V.M. Gualti and A.A. Larson. Dept. of Veterinary Biology and Program in Neuroscience, Univ. of Minnesota, St. Paul, MN 55108

Substance P (SP), a putative neurotransmitter of pain, is cleaved by peptidases in the synaptic cleft resulting in several metabolites including the N-terminal peptide SP1-7. SP1-7 has been shown to have specific CNS membrane binding sites in the male rat and DAMGO as well as biological activity including antinociception when given intracerebroventricularly in mice. To investigate SP1-7 antinociception at the spinal level, doses of 0.5, 2.5 and 20 μmol/kg were given intrathecally (i.t.) to male Swiss-Webster mice. Tail flick latency, hot plate paw lick latency or the number of abdominal stretches 5 min after an intraperitoneal injection of 0.3 ml of 1% acetic acid was determined 3, 5, 10 or 30 min after i.t. SP1-7. SP1-7 was not significantly antinociceptive (p>0.05) with the tail flick latency. A dose of 0.5 μmol of SP1-7 significantly increased the hot plate rear paw lick latency (p<0.05) at 3 and 30 min. post i.t. injection. Doses of 5, 50 and 1000 μmol/kg produced significantly decreased (p<0.05) the number of abdominal stretches 30 min post i.t. injection. To determine if opiate receptor subtypes were involved, mice were injected i.t. with 2 μg of the non-NMDA receptor antagonist, 6-cyano-7-nitrosoguanidine. These experiments demonstrate that SP1-7 can attenuate pain transmission at the spinal level through the β-FNA-sensitive opiate receptor subtype. (Supported by the Arthritis Foundation and NS21697).
SPINAL MUSCARINIC TYPE 2 RECEPTORS MEDIATE THE ANTIINOCICEPTION INDUCED BY N-METHYLCARBACHOL IN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS (PPTg).
E.T. Iwamoto, L Marion*, and J.R. Holtman. Pharmacology Dept., Univ. of Kentucky Col. of Med., Lexington, KY 40536

N-methylcarbachol (NMC) microinjection into the PPTg produces antinociception as assessed by the hot-plate and tail-flick tests. Adult male Sprague-Dawley rats were implanted with both 25 ga guide cannula aimed at the PPTg and intrathecal PE10 catheters terminating in the lumbar enlargement. One week after surgery, rats received an intrathecal injection of 0.1 μl of drug in 10 μl phosphate buffer 12.5 min before the injection of 40 nM NMC in 0.5 μl buffer into the PPTg. NMC produced hot-plate and tail-flick antinociception beginning 25-35 min which was not accompanied by losses of corneal or righting reflexes, a placing reaction, or a negative geotaxic response. Intrathecal (-)-scopolamine or methoctramine did not alter baseline nociception when administered alone intrathetically. The data indicate that either a local or a ascending spinal muscarinic M2 system mediates the antinociception produced by nicotinic stimulation of the PPTg. (Supported by NS 28847 and the KTRB.)

THE NUCLEUS RAPHE MAGNUS. L.F. Fitzgerald and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Previous studies have suggested that the pedunculopontine tegmental nucleus (PPTg) is involved in the descending control of nociception, probably through a connection to the nucleus raphe magnus (RMg). For example, electrical stimulation of the PPTg in the lightly-anesthetized rat produces antinociception in the tail flick test. The present experiments were designed to determine whether antinociception produced by stimulation of the PPTg is mediated by cholinergic receptors in the RMg. The results of these experiments demonstrate that microinjection of atropine, a muscarinic antagonist, or mecamylamine, a nicotinic antagonist, into the RMg blocked the antinociception produced by PPTg stimulation. In addition, injection of methacholine, a muscarinic agonist, or nicotine into the RMg produced antinociception. These results suggest that both muscarinic and nicotinic receptors within the RMg mediate the antinociception produced by PPTg stimulation. Supported by USPHS grant DAO8880.

NICOTINE INDUCED ANTIINOCICEPTION IS MEDIATED BY SPECIFIC CHOLINERGIC AND OPIOID RECEPTORS. M.E. Wevers, A.K. Rattan and G.A. Tejwani*. Ohio State University College of Medicine & College of Nursing, Columbus, OH 43210.

The objectives of this study were to investigate the role of nicotinic, muscarinic and opioid receptors in nicotine induced antinociception in adult male Sprague-Dawley rats. Antinociception was measured by tail-flick and hot plate 52°C tests after subcutaneous or lateral intracerebroventricular nicotine injections. Nicotine (10-1000 μg/kg, subcutaneous) or (50-200 μg/kg intracerebroventricular) induced a dose dependent increase in antinociception in both tests. Antinociception produced by subcutaneous nicotine was antagonized by mecamylamine, a nicotine receptor antagonist but not by scopolamine, hydrobromide, scopolamine methyl nitrate or hexamethonium. Opioid receptors antagonists naloxone, methylnaltrexone blocked subcutaneous nicotine effects completely and δ receptor antagonist 8-Funaltrexamine blocked subcutaneous nicotine effect only in hot plate while μ receptor antagonist WIN 44.441-13 (WIN) blocked subcutaneous nicotine effects in both tests. Antinociception produced by intracerebroventricular nicotine was antagonized by mecamylamine, hexamethonium, scopolamine hydrobromide and scopolamine methyl nitrate as well as naloxone, 8-Funaltrexamine, WIN and μ receptor antagonist naltrindole in both tests. Nicotine inhibited binding of opioid ligands to receptors in the cortex with IC50 of 2.3 μM (δ-receptors), 2.6 μM (μ-DAGO), 3.4 μM (μ-DHET) and 12.9 μM (μ-EKC). It is concluded that nicotine induced antinociception is mediated by μ, δ and κ receptors in addition to nicotinic and muscarinic receptors. (Supported by USPHS SK088N00018).

MORPHOLOGICAL CLASSIFICATION OF RETINAL GANGLION CELLS PROJECTING TO THE OPTIC TECTUM IN TURTLE. D. Zhang, M.L. Ford* and W.D. Eldred. Department of Biology, Boston University, Boston, MA 02215.

There is little detailed information about particular types of retinal ganglion cells which project to specific central targets. In this study we have examined retinal ganglion cells which project to the tectum in turtle. Rhodamine crystals were applied to the optic tectum and retrograde transport carried the rhodamine back to the ganglion cell bodies. Intracellular injection of Lucifer yellow or fluoropectin revealed ganglion cell bodies. Following photoconversion using diamino-benzidine, detailed camera lucida drawings were made. More than 20 morphologically distinct classes of ganglion cells were identified based on their stratification within the inner plexiform layer (IPL), the complexity of their dendritic arborizations and the presence or absence of dendritic beads. Ganglion cells were classified with mono-, bi- or tri-stratified cells. Different subclasses of mono-, bi- or tri-stratified cells were distinguished using the location of their arborizations within the IPL. Several different classes of diffuse type ganglion cells were also distinguished by the location of their arborizations within the IPL. Different subclasses of mono-, bi- or tri-stratified cells were also distinguished by the location of their arborizations within the IPL. Other classes of ganglion cells with the same stratification within the IPL, were distinguished by having concentrations of processes within certain strata of the IPL. Other classes of ganglion cells projecting to the optic tectum in the turtle displayed a great degree of morphological diversity, which may reflect a similar heterogeneity in their functions. This research supported by EY04785 to W.D.E.
DECREASE IN THE NUMBER OF LARGE AND MEDIUM RETINAL ADULT FERRETS. T.J. Drolsum* and E.H. Polley. Dept. of degeneration in retinal ganglion cells (RGC) in 3 ferrets (Mustela putorius furo), an assessment was made of retrograde THE SAMPLING ELEMENTS. M.H. Rowe, Neurobiology Program, Ohio TRACELLULARLY TO OTHER PARASOL CELLS, AND TO AMACRINE W ashington, Seattle, WA 98195. THURSDAY PM RETINA AND PHOTORECEPTORS: GANGLION CELLS AND CENTRIFUGAL CONTROL 1375 cell. We have applied the approach to cells in the ganglion cell layer of an in vitro whole-mounted preparation of the macaque retina, using 0.1% Thionin. Corresponding regions of the retinae were localized and the cells were counted utilizing a Camera arrays potentially results in a loss of spatial information, and this has been taken as evidence that the two subvarieties form independent sampling arrays. The relative importance of this 'position' filter can be determined directly from the statistics of the nearest neighbor histogram (French, et al., 1977). The relative bandwidth of these 'position' filters can be determined by comparing its spatial bandwidth to the Nyquist limit of the array. Thus, the positional disorder within combined on- and off-center ganglion cell arrays is not sufficient to prevent them from cooperating to achieve higher spatial resolution. DECREASE IN THE NUMBER OF LARGE AND MEDIUM RETINAL PNGION CELLS FOLLOWING TRANSSECTION OF THE OPTIC NERVE IN ADULT FERRETS. T.J. Drolsum* and K.H. Polley. Dept. of Anatomy & Cell Biology, Univ. of Ill., Chicago, Ill. 60612. As a prelude to the study of retrograde transneuronal tracing, we have been trying to obtain a list of spatial information, and this has been taken as evidence that the two subvarieties form independent sampling arrays. However, the hypothesis that the positional disorder of the combined arrays is sufficient to affect the positional spatial resolution from spatial resolution that it is being realized has not been empirically tested. Positional disorder in the spacing of the ganglion cell array does not reduce resolution per se, but acts like a low pass spatial filter. By the degree of demodulation that can be determined directly from the statistics of the nearest neighbor histogram (French, et al., 1977). The relative importance of this 'position' filter can be determined by comparing its spatial bandwidth to the Nyquist limit of the array. Thus, the positional disorder within combined on- and off-center ganglion cell arrays is not sufficient to prevent them from cooperating to achieve higher spatial resolution.
DO NMDA CHANNELS CONTRIBUTE TO THE RESTING CONDUCTANCE OF RETINAL GANGLION CELLS? J. Cottone & B.C. Miller Dept. Physiology & Graduate Program in Neuroscience, University of Minn. MN 55455.

The voltage-dependent block of the N-methyl-D-aspartate (NMDA) receptor by magnesium ions is thought to prevent this glutamate receptor subtype from being active when cell membrane potential is near resting potential. The evidence for NMDA channels in retinal ganglion cells in a larval tiger salamander slice presentation displays inward currents to applied NMDA under Mg^{2+}-free conditions (V_{rev} > -70 mV), NMDA-evoked responses are not blocked by increasing external Mg^{2+}, and a 1 mM Mg^{2+} does not abolish responses at -70 mV (1 mM Mg^{2+} 30% of Mg^{2+}-free response; n=40). These data demonstrate that NMDA currents are generated at the resting potential.

We used fluorescent coupling dyes to tag the dendritic arbors of two nontagged retinal ganglion cells in the cat retina (Torre and Poggio, 1978). We found that the inhibitory process works at the dendritic tree of the directionally selective (DS) ganglion cell (Borg-Graham and Grzywacz, 1991). Alternatively, a recent model postulates that this inhibitory process acts on the dendritic somata of the DS ganglion cell (Borg-Graham et al., 1991). A model for this computation postulates that the inhibitory process works at the dendritic endings. The output of these dendrites DS.

In this model, the dendrites receive distributed excitatory and inhibitory inputs and output the ganglion cell via the dendritic endings. The output of these dendrites DS.

We used fluorescence microscopy to image the distribution of synaptic input onto the dendritic arbors of two physiologically identified retinal X-cells. Similar data for the two cells currently being reconstructed will compare the distribution of bipolar and amacrine cell input among X-, Y-, and W-cells to determine whether the proportions of amacrine and bipolar cell input contribute to the functional differences among these retinal ganglion cell types. Supported by NIH grants EY 04777 and EY 05869.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
CIRCADIAN RHYTHMS IN THE JAPANESE QUAIL RETINA
Institute for Sensory Research, Syracuse University, Syracuse, New York

Retinal sensitivity of the Japanese quail exhibits a circadian rhythm. When the animal is held in constant darkness, retinal sensitivity as measured by the ERG b-wave, increases at night and decreases during the day (Uchiyama et al., Neurosci., Abstr., 16, 1333, 1990). Spectral sensitivity also shifts with time of day. Maximum spectral sensitivity is about 500 nm at night reflecting rod-dominance and about 600 nm during the day reflecting cone-dominance. Thus the change in rod-cone dominance of the retina (Purkinje shift) is controlled in part by an endogenous circadian clock.

What is the origin of the circadian changes in the retina? To investigate this question we analyzed both the photoreceptor (a- and b-wave) and the bipolar cell (b-component of the ERG) and found that only the b-wave exhibits a circadian rhythm in amplitude and spectral sensitivity. The spectral sensitivity of the a-wave is broad and does not change with time of day. We tentatively conclude that the circadian clock does not modulate photoreceptor sensitivity directly but rather influences the pathways that carry those signals to the inner retina.

Retinal levels of melatonin and dopamine have been found by others to undergo circadian changes in galloiform birds. We are currently investigating whether those neuromodulators have a role in the circadian rhythm of the quail retina.

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UV Light Modulates the Retinal Sensitivity of Limulus.
E.D. Herzog and R.B. Barlow, Jr.
Institute for Sensory Res., Syracuse Univ., Syracuse, NY 13244

Ultradete light enhances the circadian rhythms in the sensitivity of the Limulus lateral eye (Westerman and Barlow, 1983). Ambient UV light is sensed by a pair of frontal eyes called median ocelli. At night, this UV information is transmitted by optic nerve activity to a circadian clock located in the brain. The ocular input enhances the clock's neural output. Efferent optic nerve fibers feed the clock's output back to the ocelli and lateral eyes enhancing their sensitivity. As a result, retinal sensitivity is 0.5 log units higher at night under UV illumination than in complete darkness.

Nighttime UV illumination of the ocelli enhances retinal sensitivity; daytime illumination does not. Exposing the ocelli to environmental light at any time during the night increases sensitivity of the lateral eyes within minutes. Blocking UV illumination (<430nm) of the ocelli at night decreases lateral eye sensitivity while blocking visible wavelengths (>360nm) has no effect.

The ocular influence on retinal sensitivity may have a role in the animals' nocturnal mating behavior which is known to involve vision. Both starlight and moonlight contain significant levels of UV light. At night, sufficient amounts of UV light can penetrate the water column to a depth of 10 meters to stimulate the median ocelli. Knowing all this, we still do not understand what role UV light may have in the animal's behavior.

(Supported by NIH EY-0067 and NSF BNS 9012069)

CENTRIFUGAL INPUTS ENHANCE VISUAL RESPONSES OF RETINAL GANGLION CELLS WITHOUT CHANGING THEIR SPATIAL CODING PROPERTIES IN THE JAPANESE QUAIL.
H. Uchiyama 1,2 and R.B. Barlow, Jr. 1 1 Institute for Sensory Research, Syracuse University, NY 13244-5290; 2 Department of Anatomy, Nippon Medical School, Tokyo 113, JAPAN

Stimulation of the centrifugal fibers enhances visual responses of the retinal ganglion cells (RGC) of birds. The effect has been attributed to disinhibition, that is reduction of the inhibitory surround of the receptive field of the RGC (Miles, 1972). The inhibitory surround is thought to contribute significantly to the contrast sensitivity of the RGC, and thus disinhibition of the surround may decrease contrast sensitivity. However, a behavioral study showed that transection of the centrifugal fibers projecting to the retina does not cause any major change in visual acuity of the pigeon (Knippling, 78). To further investigate this problem, we studied the effects of the centrifugal inputs on the spatio-temporal properties of the RGC receptive fields of the Japanese quail. Urethane-anesthetized, curarized animals were used. Spatial frequency analysis showed that stimulation of the centrifugal fibers enhanced the responses of > 60% of single optic nerve fibers without changing the shape of their spatial frequency turning curve. Centrifugal inputs also changed the temporal response properties of some units. The present results do not support the conclusions of the previous studies, but suggest that the centrifugal inputs enhance visual responses of the RGC through the neural circuits that are independent of the center-surround organization of the receptive field.

(Supported by NIH EY-0067 and NSF BNS 9012069)
550.3 STRUCTURES OF THE CAT. J.K. Harting, B.V. Updike, and D.P. Van Leishout. Department of Anatomy, University of Wisconsin, Madison, WI 53705, and Department of Anatomy, Louisiana State University, New Orleans, LA 70112.

The possible significance of the patchy, mosaic-like organization of afferents to the superior colliculus is influenced by the fact that the caudal superior colliculus is slowly developing. One particularly fruitful approach has involved comparing the spatial relationships of various tectal and trigeminal afferents in younger and older animals. In the cat, the superficial colliculus is most important to the cervical system, and the deep colliculus is most important to the visual system. The general theme of these light microscopic studies has been that functionally related afferents end in patches, tectal laminae or areas, and where many afferents end, there are corresponding neurons in sublaminae or in the same laminae. In many instances, the patchy distribution of forty-four corticocollicular projections has not been found in younger animals, the visuotopy appears to be established prior to birth. Although the term inations of these projections to the superficial colliculus (SC) became responsive before any other; in particular regions of the SC became responsive to visual stimuli. Although few responsive neurons were found in younger animals, the visuotopy appears to exist prior to the caudal half of SC had higher level at birth than adulthood. AChE in LGN and Pul peaked early (day 7) but fell rapidly to adult levels, whereas SC peaked late (day 28), with a significant non-cholinergic role of AChE in early postnatal life.

Our findings reveal that fourteen cortical areas project exclusively to the SC. All of these cortical areas innervate limited regions of the SC, and the great majority end in patches. One particularly interesting finding is that several corticocollicular systems target what we have termed the mid-lower tier of the SC (Harting & Van Leishout, 1991). Such cortical input arises from SIV, SV, lateral frontal eyefields, cingulate cortex, and area 4. We have previously shown that nigral and trigeminal inputs also end in this tier, but in a complementary, nonoverlapping fashion. At this time we are investigating the specific spatial relationships of several of these “middle tier” corticocollicular systems to each other, and to various subcortical structures.

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550.5 CORTICOSTRIATAL AND CORTICOTECTAL PROJECTIONS FROM THE CAT LATERAL SUPRASYLVIAN CORTEX. J.G. McHaffie, B.E. Stein and M. Norita. Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298, and Department of Anatomy, University of Medicine, Niigata 951, Japan.

The relationships between the visual cortex and the striatum (ST) of the cat is poorly understood. The present experiments were an attempt to determine if regions along the lateral suprasylvian cortex (LS), known to project to the superior colliculus (SC) also project to the ST, and if so, to determine whether corticostriatal and corticocollicular axons arise from the same neurons. Injections of the anterograde tracer biocytin into the posterior portion of LS resulted in dense label in both ST and SC. In ST, labeled fibers and terminals were found predominantly in the caudal half of the lateral caudate nucleus and the caudal portion of the ipsilateral putamen. These cortical injections also resulted in label in the superficial and deep laminae of the ipsilateral SC. After paired injections of retrograde fluorescent dyes into ST and SC, numerous labeled LS neurons were observed in lamina V and modest numbers in lamina III; whereas, corticostriatal neurons were found in laminae I and V, corticocollicular neurons were only seen in laminae II and VII. Although labeled neurons from each injection were intermingled in lamina V, very few were double-labeled. These data suggest that while ST and SC receive substantial visual inputs from the same cortical area, the nature of that information may be quite different. Supported by NEI grant EY05554.


To evaluate the postnatal ontogenesis of corticostriatal and corticocollicular projections from the lateral suprasylvian cortex (LS), we injected the anterograde tracer biocytin into LS in neonatal cats aged 1-60 days postnatal (dpn). At the light microscopic level, both corticofugal pathways were already present at birth. Although the terminations of these projections appear to have topographies approximating those of adults, the corticostriatal population as a whole projected bilaterally, whereas the corticocollicular population was more dense on the contralateral side. The projections of the LS to the ST and SC peaked late (day 28) and fell rapidly to adult levels, whereas SC peaked early (day 7) but fell rapidly to adult levels. This is probably due to the fact that the LS is part of the visual system and is involved in controlling eye and axial body movements. These results support the hypothesis that the corticostriatal pathway forms early in development and is involved in controlling eye and axial body movements, whereas the corticocollicular pathway forms later in development and is involved in controlling eye and axial body movements.

Supported by NEI grant EY06562.


The postnatal maturation of cholinergic enzymatic activity was determined by radioautographic assay in select cortical and subcortical visual structures of the neonatal cat. CHAT activity increased progressively from birth except for a transient decrease after day 28. Adult CHAT activity was similar between the following pairs of structures: superficial and deep layers of SIV, SV, lateral geniculate (LG), and deep SC and pulvinar (PuL), area 17-18 and lateral suprasylvian cortex (LS). AChE activity increased after birth then decreased gradually to adult levels in all structures except visual cortex, which had a flat profile, and PuL, which had a slightly higher level at birth than adulthood. AChE in LGN and PuL peaked early (day 7) but fell rapidly to adult levels, whereas SC peaked late (day 28), declining gradually to adult levels. The caudal half of SC had higher activities in both superficial and deep layers, beginning at about day 14 for CHAT and day 42 for AChE. Decreasing AChE/CHAT ratios are consistent with a significant cholinergic role of AChE early postnatal life.

Supported by Grants EY06626 & EY03838.

550.8 PHYSIOLOGICAL MATURATION OF THE VISUAL TOPOGRAPHY IN THE CAT SUPERIOR COLLICULUS. C.-Q. Kao, J.G. McHaffie, M.A. Meredith and B.E. Stein. Departments of Physiology and Anatomy, Medical College of Virginia, Richmond, VA, 23298.

Anatomical and behavioral studies indicate that central visual fields are likely to develop before peripheral ones. The present experiments were initiated to determine the physiological maturation of the visuotopy in the superior colliculus (SC). Single and multunit responses were recorded in urethane-anesthetized kittens (7-50 dpn). A systematic exploration of the SC at about the time of eye opening (5 dpn) revealed topographically organized, visually-responsive neurons in superficial layers. Although fewer responsive neurons were found in younger animals, the visuotopy appears to exist prior to eye opening. There was no evidence that neurons in a particular region of the SC became responsive to any other; this may be due to the fact that most deep layer neurons were responsive to visual stimuli. These data contrast with observations that early visual behaviors depend first on the central retina. Supported by NEI grant EY06626.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
550.0  IDENTIFICATION OF CELLS IN THE SUPERIOR COLLICULUS SUBJECT TO WIDESPREAD INHIBITION.
A winner-take-all circuit must exist somewhere between the visual and oculomotor systems. We have proposed (Douglas & Vetter, 1986) that an inhibitory circuit capable of selecting one focus of activity lies in the SC. To identify which cells may be involved, iontophoretic application of glutamate or acetylcholine was used to increase the discharge levels of SC neurons in urethane-anaesthetized rats. Only cells lying in the intermediate layers showed strong inhibition to electrical stimulation of the contralateral SC. This inhibition could be blocked by bicuculline. Many of these cells could be activated antidromically from the brainstem. In addition, brainstem stimulation produced inhibition but also revealed that many projection neurons were insensitive to glutamate. The results are consistent with target selection being the last processing step in the SC.

550.11  THE EFFECTS OF SEROTONIN AND ITS ANALOGUES UPON VISUAL NEURONS IN THE HAMSTER’S SUPERIOR COLLICULUS.
X. Huang and R. D. Mooney. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699-0008
Supported by NIH grants NS 08902 and NS 22543.
Serotonin (5HT) agonists and antagonists have been shown to modify the responses of visual neurons in several species, yet the effects of these agents on single cells recorded in the hamster’s superior colliculus (SVC) are not known. We tested the effects of several 5HT agonists and antagonists on 93 units in the dorsolateral, ventral, and intermediate regions of the SVC. These responses resemble those of pigeon nBOR neurons with respect to direction preference, but were often less discriminating. For a smaller number of units, we tested the effects of 5HT: 8-OH-DPAT suppressed 43% (3/7) of cells tested and 5CT showed no effect. No differential effects on cells with particular receptive field properties were seen. The lack of differential effects may be due to the lack of cell-specific binding sites for these agonists or antagonists. These effects were attenuated by the adenosine antagonists caffeine and DIPX (100-200 μM), and by the 5HT2 blocker ketanserin. These results suggest that agonists and antagonists on retinotectal transmission in an in vitro preparation of goldfish brain.

Proopiomelanocortin-related peptides (POMC) are neuropeptides found in the retina and CNS. POMC neurons acts as an inhibitory interneuron in the retina and the CNS. We sought to determine if POMC neurotransmission on retinotectal transmission. We tested the effects of adenosine antagonists caffeine (250 μM) and 8-CPT-NECA (10 μM). Caffeine blocked the suppressive effect of 8-OH-DPAT on 5HT. The 5HT2 agonist 5m-trifluoromethyl phenyl-piperazine (5m-FMPP) had no effect on visual activity of 3 cells tested. Antagonists for 5HT2 and 5HT3 receptors, spiperone and mianserin blocked the suppressive effects of 5HT in all cells tested (n=8: 4 each agent); while ketanserin, a 5HT2 blocker, was ineffective. These results suggest that POMC may be involved in retinotectal transmission.
550.15

SOME NEURONS IN THE NUCLEUS ROTUNDUS OF PIGEON COMPUTE TIME TO COLLISION. Y.C. Wang and B.J. Frost, Departments of Physiology and Psychology, Queen's University, Kingston, Ontario K7L 3N6, Canada.

Behavioral studies have shown that the avian tectofugal pathway, which is similar to the mammalian colliculo-pulvinar-MT pathway, is involved in processing several important visual attributes, such as color, intensity, pattern, and motion. Our previous results strongly indicated that cells in the nucleus rotundus have the ability to encode simultaneous and discrete time within the nucleus rotundus of pigeon exhibited specific visual response characteristics associated with these attributes, and about 17% of cells in the dorsal-posterior subarea of the nucleus strongly responded to a looming stimulus. In this study, standard extracellular responses from dorsal-posterior subareas, EMGs from several muscles, and EKGs were simultaneously recorded from awake birds. We have found that some neurons are not only very specific to the 3D direction of motion of a “soccer ball” like stimulus, but give their maximum response at a constant time before “collision,” even when the size of the stimulus or its velocity were varied over a large range of values. The EMGs always occurred 100-200 ms after the peak looming cell response, and the heart rate increase followed even later. When the looming stimulus was not on a collision course with the bird, the looming cell’s response was dramatically reduced and no EMGs, or increased heart rate occurred. We propose that these neurons might time to “collision” and provide information necessary for an emergency avoidance response by the bird. Supported by NSERC grant A0333 to BJF.

550.17


The feline insular cortex contains cells sensitive to visual, auditory and somatosensory stimuli. The suprageniculate nucleus (SGn), receiving the superior collicular afferents, seems to be the main subcortical source of afferents towards the insular cortex. We studied the modality specificity of extracellularly recorded SGn neurons in barbiturate anesthetized, immobilized, artificially ventilated cats. Of the 136 cells tested 109 showed unimodal sensory characteristics, 23 was bimodal and only 4 was sensitive to all 3 modalities. Visual sensitivity was found in 100 cells. These showed large uniform receptive fields, including the area centralis. No retinotopy could be detected. Somatosensory sensitivity to tactile stimulation was found in 32 cells, and that to nociceptive stimulation in 5 cells. Acoustic sensitivity was found in 24 cells. No systematic relationship was found in the anatomical position of cells sensitive to various modalities. Our results show sensory properties in SGn similar to that described in the insular cortex. This is in line with the concept of parallel processing of sensory information along a thalamo-insular-cortex route.

550.16

THE EFFECT OF POSITIONAL DISPARITY ON MOTION RESPONSES OF NEURONS IN THE ACCESSORY OPTIC SYSTEM OF CAT. B.J. Frost, Dept. Psychology, York University, North York, Ontario, Canada M3J 1P3.

It is widely accepted that the accessory optic system (AOS) provides visual signals for the control of optokinetic nystagmus (OKN). In frontal-eyed animals, the substantial overlap in the visual fields of each eye and a projection from the visual cortex gives rise to an increased incidence of binocularly responsive neurons in the AOS. Visual cortical input has been shown to mediate ipsilateral eye responses and high speed tuning, and can function independently of the contralateral eye. However, binocular tuning characteristics of AOS cells have not been extensively examined. The present study set out to determine if binocular AOS cells are sensitive to retinal disparity. Single units were recorded from the dorsal terminal nucleus (DTN) of the inferior olive, anesthetized, paralyzed cats. The positions of area centrales were determined by fundus reflection. Convergent and divergent disparities were generated by deviating the visual axis of one eye using wedge prisms ranging from ±1-20 diopters (i.e., base-in or -out, 1 diopter=0.57 deg). A random-dot pattern composed of multiple gray levels was moved by computer at a constant velocity in the preferred direction. The responses of DTN units to position disparity fell into 4 categories: 1) left- and tuning profiles similar to “sustained excitatory” responses consisting of a marked facilitation for a single or a small range of disparities (convergent and divergent); 2) cells relatively insensitive to disparity, showing a relatively flat response profile across the entire range of disparities; 3) cells displaying rather complex tuning profiles which usually contained multiple peaks or troughs at different disparity values; and 4) a small number of cells broadly tuned for inhibition. In summary, this study demonstrates that many AOS cells are sensitive to position disparity and supports behavioral investigations showing that OKN gain is dramatically affected by changes in depth planes defined by disparity. Supported by a grant from NSERC.

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551.2

INFERIOR OLIVE DISEASE IN MAN IMPAIRS LEARNING NOVEL SYNBERGIES. W.T. Thach, J.G. Keating, and H.P. Goodkin, Dept of Anatomy Neurobiology and Neurology, and the I.W. Rehak Institute, Washington University Medical School, St. Louis, Mo. 63110.

In grooming, the eye (and head) fixate the target, and serve as a reference aim for the arm. Coordination between gaze direction and arm throw is a skill: it is developed and maintained by practice. When wedge prism spectacles are placed over the eyes with the base to the right, the optic path is bent to the right, and the eyes (and head) move to the left to fixate the target. The arm, calibrated back to the left, gives a correct throw. With practice, the calibration changes, and the arm throws close to and finally on-target. Proof that eye and head position is the reference aim for the arm throw trajectory occurs when the prisms are removed and the arm throws. The eye are now on-target, but the eye-head arm calibration for the previously looming gaze fails: the arm throws to the right of target an amount almost equal to the original leftward error. With practice, eye-head position and arm synergy is reestablished: each throw falls closer and finally on-target. Bains showed in macaques that the adjustment is abolished by cerebellar lesions (Baizer and Glueckstein, 1974). Weiner et al (1983) obtained similar results in patients with cerebellar disease, and showed that adaptation was not altered in disease of corticospinal or basal ganglia systems. We have confned these results in patients with pure cerebellar cortical disease (1 infant, 2 cortical atrophy). We have also studied two patients with MRI-documented inferior olive hypoplasia. Both had palatal myoclonus and progressive ataxia of trunk and gait. Neither showed adaptation on this task, despite near-normal throwing performance (cf also Gauthier et al 1979). Finally, we studied patients with lesions presumed to involve motor fibers of the middle cerebellar peduncle, who also show impaired adaptation (cases of ataxia hemiparesis, with contralateral lesions of the basis pontis involving leg corticoepial and arm pontocerebellar fibers, according to Fisher, 1978). These results indicate that cerebellar cortex, inferior olive, and motor fibers are all necessary for the adaptation, but they do not distinguish the roles played by each (NHS grant NS15297).
DURING SPONTANEOUS ALTERATIONS IN LIMB COORDINATION.

551.7

the night of behavioral estrus following endogenous increases in estradiol and limb coordination. Limb response to changes in treadmill speed is most accurate on with errors in placement or transitions in terrain. Increases (42-74%) in cerebellar cell discharge is correlated with limb movement, while olivary discharge is correlated employing (L) constant speed (4 or 11 cm/s) or ii.) variable acceleration. Typically, Pnj neurons, simultaneously. Neuronal activity was monitored during treadmill paradigms recorded from Purkinje (Pnj) cells of the paravermal cerebellum and inferior olivary in addition, olivary activity tested on estrus during variable acceleration, discharges and decreases (15-24%) in olivary discharge correlated with treadmill movements, both groups in initially showed increased error. Normal movements, both groups initially showed increased error. Normal subjects then improved rapidly, while patients showed only gradual improvement. When fast movements were requested first, patients improved less rapidly than normals. Errors in patients correlated with mean square part of the trajectory.

Patients with cerebellar deficits can exhibit normal rate of skill improvement. When fast movements were requested first, patients and controls reduced the error over trials. Rate of improvement was similar. When starting the fast movements, both groups initially showed increased error. Normal subjects then improved rapidly, while patients showed only gradual improvement. When fast movements were requested first, patients improved less rapidly than normals. Errors in patients correlated with mean square part of the trajectory.

Patients with cerebellar deficits can exhibit normal rate of skill improvement. When fast movements were requested first, patients and controls reduced the error over trials. Rate of improvement was similar. When starting the fast movements, both groups initially showed increased error. Normal subjects then improved rapidly, while patients showed only gradual improvement. When fast movements were requested first, patients improved less rapidly than normals. Errors in patients correlated with mean square part of the trajectory.
551.0
CEREBELLAR CONTROL OF PROPRIOCEPTIVE A NEW PARADIGM M. Gorassini and A. Prochazka, Division of Neuroscience, Univ. of Alberta, Edmonton, Alberta, T6G 2Z5, CANADA.

It is thought that one of the roles of the cerebellum is to control the gain of sensorimotor transmission in extracerebellar reflex pathways, in particular, the proprioceptive system. Sherrington has called the cerebellum the "brachial ganglion" of the proprioceptive system. We wish to test the hypothesis that the cerebellum controls the stretch sensitivity of the spindle receptors by influencing fusimotor output. Perhaps abnormalities in the control of proprioceptive gain contribute to the ataxia and tremor seen in cerebellar disease. To test this, we have developed a preparation that will allow us to record muscle afferent discharge during reversible inactivation of the cerebellar cortex in the freely moving cat. An injection port chronically placed over the paravermal cortex acts as a probe for the injection of 100 μl of 2% lidocaine onto the cortical surface. Recording microelectrodes are inserted into the L7 dorsal root ganglion and single unit activity is transmitted by telemetry. To date, we have implanted two cats and have observed both, marked ataxia and large postural deficits in limbs ipsilateral to the injection site. These deficits are reversible and temporary, lasting for only 3 to 5 minutes on average. If fusimotor abnormalities contribute to this ataxia, we will be able to monitor this in the afferent recordings. In support of this, we have observed a marked increase in spindle discharge upon injection of lidocaine and cooling of the cerebellar cortex in two acute experiments. (Supported by MRC of CANADA.)

CEREBELLUM III

552.1
SITES OF MOTOR LEARNING IN THE VESTIBULO-OCULAR REFLEX (VOR) PREDICTED BY A DYNAMICAL NETWORK MODEL. T.J. Sejnowski and S.G. Lisberger. The Salk Institute, La Jolla, CA 92037, and Department of Physiology, University of California, San Francisco, 94143.

A dynamical network model of the VOR and smooth pursuit eye movements was used to explore possible sites of plasticity of the VOR. The model had processing units that were nonlinear leaky integrators interconnected in a pattern based on the known anatomy of the VOR pathways. There were two vestibulo-motor pathways: a direct one through the brainstem and an indirect one through the cerebellar flocculus. The flocculus also received negative visual feedback related to image velocity and positive feedback related to output eye velocity. We used a gradient descent optimization procedure to adjust the parameters of the model so it achieved good tracking during VOR and smooth pursuit trials and ii) to determine how the network would reduce the amplitude of the VOR while maintaining excellent pursuit.

Reductions in the amplitude of the VOR in the model were achieved by decreasing the connection weights in the vestibular input to the brainstem. The connection weights in the vestibular input to the flocculus increased during the early stages of learning, as suggested by M. Ito, but decreased during the later stages, as demonstrated by F. Miles et al. Because gradient descent optimization allowed learning at all sites in the model, we conclude that the pattern of weight changes resulted from other factors, such as the anatomical structure of the network and the requirement for accurate dynamic tracking during visual and vestibular stimulation. Similar factors may determine the sites of local learning mechanisms in the VOR pathways in the brain. (Supported by DARPA through the Office of Naval Research and the Howard Hughes Medical Institute.)

552.2
A NETWORK MODEL OF THE CEREBELLUM THAT USES A TRAINED SET OF PATTERN GENERATORS TO CONTROL A SINGLE DEGREE-OF-FREEDOM JOINT. R.E. Berthier, A.G. Bartis and J.C. Hesl, Dept. of Computer and Information Science, Univ. of Mass., Amherst, MA 01003.

Recently we proposed a model of motor control that considers the cerebellum as a distributed pattern generator. The selection and a set of APGs is determined by the postsynaptic effects of propriospinal and tectospinal parallel fibers that convey information about the internal and external state of the organism. The response of an APG to a target input is adaptively altered by the action of climbing fibers which are seen as providing private training signals evaluating the APG's contribution to movement.

The current research is an extension of previous work where APGs were trained to control movements of a planar, kinematic arm. In that work a physiologically inspired learning rule was used to adapt parallel fibers conveying target information to a Pachyneur cell. Because only one target line was active at a time, this was equivalent to adapting a single parallel fiber per trial. In the present experiments, we sought to extend the learning rule to more realistic situations where all parallel fiber inputs to an APG are adapted and where APGs must act cooperatively to control a single degree-of-freedom joint with nonlinear muscle dynamics. Simulations were performed in which parallel fiber inputs provided redundant, irrelevant, or distracting information. In these simulations, a subset of the APGs could be trained to move the joint to one target, and a different subset trained to move the joint to another target of greater joint angle. (Supported by NSF R00014-68-E0309 and the McDonnell-Pew Foundation for Cognitive Neuroscience.)

552.3
COMPUTATIONAL MODELS OF THREE REGIONS OF THE CEREBEL­LUM M. Kawato and H. Gum, ATR Auditory and Visual Perception Research Laboratory, Amakusa, Kyushu, Nippon 869-01 Japan.

In supervised motor learning, one of the most essential and difficult problems is how to convert the error signal measured in the sensory space (trajectory error in the task-oriented coordinates) into the error signal for the motor command in the coordinates of muscles. We proposed the feedback-error-learning approach where the feedback motor command is used as an error signal to train a neural network which then generates a feedforward motor command. The cerebellum is divided into three functionally distinct parts: the vestibulocerebellum, the spinocerebellum, and the cerebrocerebellum with distinctive anatomical connections although the cellular organization of the cerebellar cortex is simple, regular and uniform. The feedback-error-learning neural network was originally proposed as a model for the cerebrocerebellum. The spinocerebellum receives sensory information from the periphery. We proposed a closed loop control system based on the feedback-error-learning as a model of the spinocerebellum. The vestibulocerebellum sends a signal to the DSCT which activates a command for a single degree of freedom joint. The feedback-error-learning neural network was trained to control movements of a planar, kinematic arm. In that work a physiological inspired learning rule was used to adapt parallel fibers conveying target information to a Pachyneur cell. Because only one target line was active at a time, this was equivalent to adapting a single parallel fiber per trial. In the present experiments, we sought to extend the learning rule to more realistic situations where all parallel fiber inputs to an APG are adapted and where APGs must act cooperatively to control a single degree-of-freedom joint with nonlinear muscle dynamics. Simulations were performed in which parallel fiber inputs provided redundant, irrelevant, or distracting information. In these simulations, a subset of the APGs could be trained to move the joint to one target, and a different subset trained to move the joint to another target of greater joint angle. (Supported by NSF R00014-68-E0309 and the McDonnell-Pew Foundation for Cognitive Neuroscience.)

552.4

We present a new description of the functional organization of the dorsal spinocerebellar tract (DSTC) which accounts for the extensive polysynaptic component of its behavior. A reevaluation of DSTC function was required by several recent findings including: 1) a profound influence of interneurons on DSTC activity evidenced by the polysynaptic activation of 80%-90% of the DSTC by afferents from a single muscle, and 2) a widespread convergence of afferents from muscle, joint and skin onto interneurons projecting to the DSCT.

We show in a representative population of DSTC neurons in cat that the activity of individual cells is affected by diverse sensory receptors activated by hindlimb movements. Population responses are characterized by known responses to specific sensory inputs which is known to be the convergence of climbing fibers to the DSCT. We found no evidence for distinct presynaptic pathways or functional cell types. These data are consistent with a model for the DSTC having a parallel distributed presynaptic circuitry. According to the model, DSTC activity results from a weighted distribution of inputs onto an extensive interneuronal network projecting to DSTC neurons. (Supported by NIH Grant NS 21143.)
552.5 A METHOD OF EXTRACELLULAR MICROSTIMULATION WITH COINCIDENT RECORDING. J.D. Renthal, Univ. of Ill. Coll. of Med., Rockford, Illinois 61107.

To record and stimulate in the extracellular field with one electrolyte-filled micropipette (3M NaCl) requires that sub-millisecond signals be visible <0.5 ms after passing several μA. Major problems are capacitive stimulus artifact and transient tip potentials from the electronic switching between current source and amplifier input, which leaves a tolerable if inconvenient switching artifact. TTFs, which reach 1-20 mV and last >2 ms, were shown to result from depletion of ions entering the microelectrode, and to vary as the square of the measured impedance. Pressure ejection of 3M NaCl and differential recording and stimulation with matched microelectrodes (one in brain, one elsewhere) minimized them. Several discretely recruited spikes were usually detected 0.5 ms after rectangular stimulus pulses (1-3 μA) in the cerebellar cortex of unanesthetized rats. All-or-nothing properties and refractoriness within 2-5 ms were evident for the spike of lowest threshold. Longer, complex waveforms were also sometimes found. The technique offers a global search stimulus and improved method of functional brain mapping. (Supported by NINDS grant 26116.)

552.6 TEMPORAL RELATIONSHIPS BETWEEN CEREBRAL CORTICAL AND CEREBELLAR RESPONSES TO TACTILE STIMULATION IN THE RAT. J. Mortenite, M. Lee, and J. M. Bower, Computation and Neural Systems Program, California Institute of Technology, Pasadena, CA 91125.

Cerebellar granule cell responses to brief (10 msec) tactile stimuli consist of an initial component with a short 8-10 msec latency and a second component with a more variable latency of 16-32 msec. We have recently shown that the longer latency component of this response is much more sensitive to neonatal lesions than is the shorter latency component (Mortenite et al., Soc. Neurosci. Abstr. 16, 370.1, 1990). Accordingly, we were interested in determining the pathway responsible for this longer latency cerebellar response.

Several different experimental techniques were used to demonstrate that the long latency granule cell response component is dependent on a pathway involving the somatosensory (SI) cortex. Idiosepic injections into SI, local SI ablations, and complete midcollicular sections all substantially interfere with the second component of the cerebellar response without having any effect on the short latency response. Further, when tactile evoked responses were simultaneously recorded in both the cerebellar granule cell layer and layer IV of SI cortex, the latencies of both responses were strongly correlated. Intraperirenal injections of sodium pentobarbital during these recordings produced a highly correlated increase in latency in both the SI and the second component of the cerebellar response. These results suggest that the topologically organized SI projection to the cerebellum (Bower et al., Brain Behav. Evol. 18, 1, 1981) influences cerebellar cortex at late and variable latencies. Dual recordings are currently being used to investigate the influence of SI cortex on the late and variable latency cerebellar fiber responses to tactile stimulation.

Supported by NIH (NS22205) and BRSG grant (RR70003).

552.7 TEMPORAL AND SPATIAL ORGANIZATION OF CLIMBING FIBER TACTILE RESPONSES IN RAT CEREBELLAR CORTEX. M. Lee and J. M. Bower. Divisions of Biology 216-76 and Biology 216-24, Caltech, Pasadena, CA 91125.

The climbing fiber (CF) projection to the cerebellum mediates Purkinje cell complex spike responses to tactile stimulation. The temporal and spatial organization of these responses was studied with reference to the well-characterized map of tactile responses in the granule cell (GC) layer of folium crus 2a in rat cerebellar cortex (Bower & Kassel, J. Comp. Neurol. 302:768, 1990). Temporal organization. CF responses to tactile stimulation are sparse, occurring only about 1/5 of the time for regular (1 Hz) punctate stimulation, as seen in dot-raster displays of spike data. This characteristic appears to be related to the 10 Hz periodicity of CF activity (Sasai et al., Eur. J. Neurosci. 1:572, 1989). Since stimulation during "off" phases of the excitability cycle have a much lower probability of producing a spike response. CF responses also occur at latencies later than those of GC multisensory responses to the same stimuli, as seen in PST histograms of spike data. CF response latencies are around 50 ms, as compared to 2-5 ms for GC responses. The CF response often involves a rebound spike at 150 ms. These CF response latencies are, however, on the same order as those of GC-mediated increases in Purkinje cell simple spike activity in response to tactile stimulation (Thompson & Bower, this volume).

Spatial organization. CFs with similar tactile receptive fields are arranged in a "patchy mosaic" that is roughly register with the underlying map of GC receptive fields. Thus CFs with non-contiguous receptive fields can be located close to each other in the cerebellar cortex. However, CF receptive fields are large, typically several times larger than GC receptive fields, and bilateral, even when receptive fields in the underlying GC layer are unilateral. For example, in the center of crus 2a, GCs have receptive fields on the ipsilateral upper lip, but CFs have receptive fields covering both sides of the upper lip. Supported by NIH grant NS 22205.


For the last several years we have been exploring our knowledge of the detailed pattern of tactile receptive field projections to the granule cell layers of crus Ila to explore the physiological relationships between granule cells and Purkinje cells. Specifically, using in vivo extracellular recording techniques, we have monitored Purkinje cell activity in crus Ila of the rat while applying very brief (5 msec) tactile stimuli to perriform regions known to project to underlying or nearby granule cell layer locations. Our results show that Purkinje cell responses to these brief peripheral stimuli do consist of a short latency component (5-8 msec) that but that can also be produced a prolonged (100-400 msec) increase in simple spike activity. By comparing the latencies between simple and complex Purkinje cell responses we have been able to show that climbing fibers are also activated by these stimuli at latencies of around 50 msec (Lee and Bower, this volume). However, when those trials in which climbing fiber responses do occur (about 1 in 5) are separated from those that have only simple spike responses, the prolonged simple spike response is still present. This result suggests that the mossy fiber / granule cell system is capable of generating prolonged changes in Purkinje cell activity in response to natural tactile stimulation.

We have pursued this result using both in vivo intracellular techniques (Jaeger and Bower, this volume) and detailed single Purkinje cell modeling (De Schutter and Bower, this volume).

Supported by NIH grant NS22205.

552.9 A COMPUTER SIMULATION OF PLATEAU POTENTIALS AND SYNAPTIC INTERACTIONS IN PURKINJE CELL SPINY DENDRITES. E. de Schutter and J. M. Bower. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

EM-reconstruction of granule cell to Purkinje cell synapses has shown a spatial segregation between parallel fiber synapses and synapses from the ascending part of the granule cell axon (Tapp-Sullar & JMB, Abstr. Soc. Neurosci. 16: 896, 1990). The position of parallel fiber synapses between the ascending branch synapses and the main Purkinje cell dendrite is consistent with a modulation of the parallel fiber segment, with the main excitatory effects coming from ascending fiber inputs from underlying granule cells. Extracellular in vivo recordings (J. Thompson & JMB., this volume) and intracellular recordings in slices (D. Jaeger & JMB., ibid.) show that granule cell stimulation generates postsynaptic plateau potentials of 100 to 400 ms in Purkinje cell dendrites.

To examine the significance of these plateau and of the interactions between ascending and parallel fiber synapses we have constructed a detailed compartmental model of a Purkinje cell, based upon anatomical measurements. The model includes sodium, calcium and potassium currents, quisqualate and GABA synaptic conductances and diffusion, buffering and extrusion of calcium. Temporal summation of plateau potentials as well as effect of increasing the timing of time-locked parallel fiber and inhibitory stellate cell synaptic activity on somatic spike firing after an ascending fiber plateau have been examined. The fast increase in internal calcium concentration after application of glutamate on dendrites, and the possible effects of cytoplasmic calcium uptake and release mechanisms have been simulated.

Supported by PHS 5 T32 NS04568 to EDH and NIH grant NS22205 to JMB.

552.10 ANALYSIS OF PLATEAU POTENTIALS SEEN IN INTRACELLULAR PURKINJE CELL RECORDINGS WITH SYNAPTIC STIMULATION. D. Jaeger and J. M. Bower. Computation and Neural Systems Program, Caltech, Pasadena, CA 91125.

For the last several years we have been exploring the effects of granule cells on Purkinje cells within the rat cerebellar cortex utilizing in vivo extracellular recording techniques (J. Thompson and J. M. Bower, this volume). Our data has suggested that purely granule cell input, even in the absence of climbing fiber activation, can generate prolonged (100-400 msec) increases in simple spike activity. In the present study we have used in vivo intracellular recording procedures to further explore these prolonged effects. Specifically, slices were cut in two orientations; paradoxically to preserve Purkinje cell inputs from the ascending granule cell axon while significantly reducing input from parallel fibers, and, horizontally, to preserve both components of the granule cell input.

Our results reveal that the synaptic activation by granule cells produces a prolonged membrane depolarization lasting 100-400 msec in both the soma and dendrites of Purkinje cells. These depolarizations can generate trains of simple spikes and can occur at long latencies. As in the in vivo preparation, prolonged responses occur with or without concomitant climbing fiber activation. A careful examination of the interaction between the climbing fiber response and the prolonged depolarization has provided critical information for modeling the locations and the properties of the conductances underlying these responses in a realistic Purkinje cell model (E. de Schutter and J. M. Bower, this volume).

Supported by NIH grant NS22205.
The encoding of the location and magnitude of climbing fiber responses to tactile stimulation of the face varied for different regions of the cerebellar cortex. An analysis of face receptive fields yielded invertebrate Purkinje cells. The results indicated that climbing fiber responses encountered in the anterior lobe were consistent across all five transport speeds in the reach was systematically increased. A subject, a below-elbow amputee, was used to investigate the role of cerebellum in the control of human movements. The results of the present study, which were consistent with previous studies, suggest that the cerebellum is involved in the control of a variety of movements, including those that are essential for reaching and grasping objects. The findings also support the idea that the cerebellum is a key component of the motor control system, and that it plays a critical role in the coordination of movements across different body parts.

For more information, please contact the authors at sue.wallace@colorado.edu or l.e.carlson@colorado.edu.
553.3 HANDEDNESS, PRACTICE ORDER, AND ASSIMILATION EFFECTS IN RAPID BIMANUAL MOVEMENT. D.E. Sherwood. Motor Behavior, Univ. of Colorado, Boulder, CO 80309.

When subjects make rapid bimanual aiming movements over different distances, assimilation effects are typically shown as overshoots in the shorter-distance limb. The effect of practice order and handedness on assimilation effects were assessed by giving 60 right-handed, 60 left-handed and 30 right- and left-handed subjects practice on 20° (Short) and 60° (Long) reversal movements first separately, then together. Subjects were randomly assigned to a practice order of either Short-Long or Long-Short and performed the Short and Long movements in either the left or right hand. For all subjects, overshooting was shown on the dual trials in the shorter-distance limb, showing assimilation effects. This suggests that assimilation effects can be influenced by practice variables.


Muscle function can be influenced by a decline in the number of motor units in a muscle and, through reinnervation, an increase in the innervation ratio for some motor units. The purpose was to determine if these changes influence the behavior of high-threshold motor units. Seventeen healthy subjects (64-79 years) volunteered for the study. We measure the contraction force curves of the flexor digitorum superficialis muscle of the left hand in 22 subjects. The EMGs were recorded using hooked wires inserted into 6 or 7 index finger muscles (extensor indicis EMG was not always recorded). Movements were recorded opto-electronically with infrared emitting diodes on the base of the middle and index finger and the centers of the 3 finger joints. Subjects performed 6 types of discrete movements involving flexion or extension of all 3 fingers with the MCP joint while the interphalangeal IP joints were voluntarily held in flexion, and flexion or extension at the PIP and DIF joints while the MCP joint was voluntarily held straight. Analysis of velocity profiles showed that there is coupling of PIF and DIF movements during flexion or extension of these joints. The EMG data showed: (1) maintenance of flexed RP or interphalangeal joint angles while moving the other joints requires coactivation of several muscles, suggesting that joint stiffness is increased to compensate for dynamic interaction torques and (3) synergistic muscle activities are coordinated differently according to the task, and exhibit higher correlations when acting as agonists than as antagonists. Supported by NIH grant ROI-AR40117


A functional relationship exists between the intrinsic joint biomechanics provided by passive tissues and the muscle activation patterns dictated by the CNS. This relationship can theoretically be quite simple—the CNS may activate muscles to produce the required torque(s) about the joint axis(es) of rotation while allowing passive tissues to equilibrate torque components in other directions. Alternatively, muscle activation patterns may be designed to reduce passive tissue loading. In this study we investigated the relationship between activation of the primary elbow flexors and the intrinsic mechanics of the elbow joint. EMGs were recorded from the muscles of the elbow using surface and intramuscular electrodes while subjects isometrically generated force against a 3-DOP load cell (flexion/extension, pronation/supination) located distally on the forearm. The first set of experiments EMGs were recorded for eight levels modulated to assist the passive tissues in equilibrating varus-valgus torques and the muscle activation patterns dictated by the CNS. This re-...
553.9
RAT SKILLED REACHING: MOVEMENT COMPONENTS AND NEUROCORTICAL ORGANIZATION Jan Q. Whishaw, Boguslaw P. Corny* and Sergio M. Pellas, Department of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 1V4.

Rats are skilled at reaching for and grasping food objects using one limb. The movement can be divided into a subcomponents. Food is localized using olfaction. Using movements of the upper arm, a limb is lifted and aimed at the food, transported to the food, and the paw is pronated over the food. The digits are opened during the transport phase and are closed after palm contact initiates the retraction phase of the movement. During retraction, the paw is supinated at the wrist in two 90° movements, one occurs during retraction and one after retraction, to present the food to the mouth. Some of these movements can be disrupted by neurocortical motor cortex area damage but whole body movements can be used to substitute for the missing reaching movements. On the basis of the behavioral results, cortical anatomy, and cortical electrophysiology, it is suggested that the different components of reaching have partially independent cortical representations. It is also proposed that some of the components or rat reaching to human reaching argue for parallel development or homology.

553.11

During sustained, submaximal, isometric contractions, motor units (MUs) in human biceps brachii muscle exhibited a diversity of discharge patterns (Garland et al., 1989). The present experiments were performed to determine if MUs have similar patterns during fatiguing elbow movements. Subjects performed 100 flexion-extension elbow movements (40°, 0.6/s), utilizing a symmetrical phase-plane tracking paradigm. A constant torque of 1.0 Nm, opposing extension, was applied to the handle moved by the subject. MU activity was recorded from the lateral head of triceps using a branched bipolar fine wire electrode inserted subcutaneously overlying the muscle fascia.

During extension, MUs were phasically active at movement onset and tonicly active at the end of the movements. The activity of MUs, which were strongly active in early trials, remained unchanged or decreased with fatigue. Other MUs, which were rarely recruited in early trials, became more active with fatigue. A given MU could be phasically active at movement onset even if its tonic activity was reduced during fatigue. Since the movement performance remained constant despite fatigue, our data suggest that the performance was preserved by the recruitment of additional MUs. These data concur with MU data from fatiguing, sustained, isometric contractions. Funded by NSERC and Academic Development Fund, UWO.

553.12

Recent studies have shown that surface electromyographic (EMG) activity is related to the acceleration - deceleration characteristics of the desired movement. We have recorded from single motor units (SMUs) in humans performing movements with specified accelerations and decelerations longer than in the tracking task. Results coincide with what has been found for smooth pursuit eye movements, suggesting that the nervous system uses similar strategies to control the initiation of both eye and limb movements.

553.13

Specific large fiber sensory neurones (LFSN) produce severe disorders of posture and movement. Recent studies (Neurosci. Abst. 14: 953) have revealed large direction-dependent asymmetries of the MU activity with forward and backward control of movement. Despite the apparent locomotor nature of the system, the directional asymmetry of MU activity was found to be constant in both walking and stepping. In the present study we asked whether proprioceptive input is also necessary for the temporal coordination of joint motions since motion at individual limb segments may produce torques at other segments. We studied the trajectories of repetitive "slicing" gestures made by a 43 y/o woman with LFSN and by 9 normal controls. Hand, wrist, elbow and shoulder movements were digitized online and input to a WATSMART system. The patient was unable to maintain the normal linearity and planarity of individual or successive movement cycles and the velocities at the different joints were temporally decoupled. A large anomalous velocity disruption at the wrist occurred at the end of shoulder flexion before shoulder extension when the intrathoracic motion in smooth pursuit eye movements. This error resulted from a lack of synchronization of shoulder and elbow joint motions. It reflected an interaction torque at the elbow which was not adequately compensated by active muscle contraction and could be phasically active at movement onset even if its tonic activity was reduced during fatigue. Since the movement performance remained constant despite fatigue, our data suggest that the performance was preserved by the recruitment of additional MUs. These data concur with MU data from fatiguing, sustained, isometric contractions. Funded by NSERC and Academic Development Fund, UWO.

553.14

Changes in motor unit EMG activity and reflex responses to cutaneous stimulation depend critically on the location of skin stimulation. We have examined the effect of cutaneous stimulation on the response of adductor pollicis (AP), first dorsal interosseous (DIO) and adductor digiti minimi (ADM) to transcranial magnetic stimulation (TMS) of the brain. The MU activity was recorded using single electrode EMG with a 9 cm coil, centered at the vertex, at a suprathreshold for a response in the relaxed muscle. In the absence of cutaneous stimulation, the MU's activity could be switched from movement initiation to movement termination by altering acceleration and deceleration magnitudes. If, however, the mean acceleration magnitude was constant, any active MU continued to be active for all acceleration durations tested. There was no evidence that changes in the duration of surface EMG bursts were produced by recruitment of new MUs late in the burst period. Rather the duration of MU firing varied with the acceleration and deceleration durations.

A given motor unit can thus be active both when its muscle is acting as an agonist and as an antagonist. When and whether the unit is active depends on the desired acceleration and deceleration characteristics of the movement. Supported by NSERC (Canada) and the UWO Academic Development Fund.
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553.15

CHARACTERISTICS OF HANDBAND CURVATURE FOR PLANAR, MULTIJOINT ARM MOVEMENTS. G.M. Kayes. Department of Therapeutic Science, University of Wisconsin-Madison, Madison, WI 53706.

There are conflicting reports in the literature regarding the kinematic characteristics of the hand path and their relationship with multijoint and limb movements. The aim of this study was to: (1) better characterize the kinematics of the hand path for a wide variety of planar reaching movements; and (2) attempt to relate those characteristics to the muscle activation patterns responsible for the movements.

Normal, adult subjects performed self-paced reaching movements in 12 different directions and from different initial limb configurations. Kinematic data were obtained using a WATSMART motion analysis system, and EMG activity was recorded from shoulder and elbow muscles involved in the movements. Hand paths were characterized by the direction and extent of the major curvature and by the number and location of inflections. These kinematic characteristics were found to vary in a consistent manner relative to the initial limb configuration and the spatial direction of movement. These direction-dependent variations in hand path characteristics were then studied in relation to initial muscle activation patterns. Changes in the direction of curvature appeared to be associated with qualitative changes in the EMG pattern (e.g., the switch from initial flexor activity to initial extensor activity at one joint), while changes in the extent of curvature and location of inflection points were associated with variations in the timing and magnitude of the initial EMG activity at the shoulder and elbow.

553.16

ARM AND TRUNK SYNERGIES IN POINTING MOVEMENTS IN NORMAL SUBJECTS AND PATIENTS WITH CEREBELLAR DEFICITS. A.M. Iannone, M. Hallett, S. Staropoli, P. Light. Human Motor Control Section, NINOS, Bethesda and MCO, Toledo.

The coordination of unconstrained multijoint movement in three dimensional space is a difficult task and can be compromised in patients with cerebellar deficits, particularly when the motor task is novel. We studied the kinematics of one such movement and compared the performance of patients with that of normals. Subjects sat on a chair and rested their right arm on a table in front of them. Pointing movements were made to targets at shoulder height and distances that were 80%, 100% and 120% of arm length. Movements were made at freely chosen "fast" and "slow" speeds. Anterior-posterior trajectories of the trunk and angles of the shoulder and elbow were measured using the Vicon System.

The movements of the different body parts was consistent for each individual for each task, but clearly changed with movements of different distance. For example, the trunk movement was backwards for the short distance, minimal for the middle distance and forward for the long distance. Movements of the trunk, shoulder and elbow were simultaneous and were coordinated to produce a nearly linear trajectory of the finger to the target. While the patients usually showed normal simultaneous movements of the different body segments, the coordination was impaired resulting in misdirected, multisegemented trajectories. These trajectories showed variable errors in the ratio of forward and vertical movement needed for a smooth trajectory. In particular the movement of the trunk in the long distance movement was abnormal as it moved twice the distance of normal subjects. The reason for this is not clear.

The coordination of these multijoint movements is characterized by similar time courses of the individual components. The coordination was impaired resulting in abnormal not because of timing errors, but by virtue of inaccurate component movements.

553.17

EFFECTS OF STATIC TORQUE LOAD ON POSTURAL ACTIVITY ASSOCIATED WITH RAPID WRIST MOVEMENT AND ARM HOLDING IN MAN. F. Aoki Department of Rehabilitation Research, Tokyo Metropolitan Institute of Gerontology, Ibaraki-ku, Tokyo 173.

Effects of static torque loading on phasic postural activity associated with rapid wrist movement were examined and compared with effects on tonic postural activity during arm holding. Subjects, holding their arm in a horizontal plane with the forearm supinate, made a rapid wrist flexion in response to a tone signal. Motor tasks were executed in 6 conditions, involving combinations of 3 loads to the wrist (flexor load, extensor load or no load) and 2 modes of support to the forearm (with or without support). Phasic activities of the forearm and upper arm muscles related to the rapid wrist flexion and tonic activities related to the arm holding were compared with each other in terms of static load effects. The tonic activities of both the forearm and upper arm muscles during the arm holding were influenced by the load condition, and activities of the upper arm muscles were affected by the support condition. In contrast to the tonic activities, the phasic activities of the forearm and upper arm muscles related to the rapid wrist flexion were affected by the load condition, but not by the support condition. These results suggest that tonic postural activities are influenced by static loading, while phasic postural activities associated with rapid movement are controlled independently of static torque condition.

553.18

OPTIMAL ARM MOVEMENTS. M. Donato, M. Kawato, Y. Uno* and R. Suzuki*. Cognitive Processes Dept. ATR, Kyoto 619-02 Japan and Dept. of Mathematical Engineering, Tokyo University, Tokyo, 113 Japan.

In order to control a voluntary hand movement, the CNS must determine: (A) which trajectory (hand path and velocity) should be used while moving the hand; (B) what motor commands should the muscles receive to specify each intermediate state of the hand while it is moving. Two main antagonistic approaches have been proposed to explain the control strategies used by the CNS: the minimum-jerk model (MJM) and the minimum-jerk model (M/M) proposes a solution only to the trajectory determination problem (problem A). An antagonistic approach, the minimum torque-change model, takes into account the structure and dynamics of the arm and predicts simultaneously both the hand trajectory and the joint torques. Both models were criticized for their inability to reproduce some experimental arm movement (Uno et al. 1989; Flash 1990). However, Uno et al. (1989) found that the MJM reproduced movements of normal human subjects quite well based on a simple 2-link 6-muscle model.

The following study is based on the belief that the structure and dynamics of the arm must play a role in the determination of hand trajectory. A detailed dynamic model of the human arm is used to investigate this possibility that minimizing the rate of change of: (A) motor commands to the muscles, (B) torque created by each muscle, (C) tension created by each muscle, (D) potential energy stored by each muscle, during a planar movement in the frontal space, can produce by itself realistic trajectories comparable to those of normal human subjects. In order to model the human arm data quite well based on a simple 2-link 6-muscle model.

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553.19


The purpose of this study was to examine how the kinematic characteristics of the reach and grasp are influenced when individuals reach towards moving targets of various velocities. Subjects were required to reach towards, grasp, and pick up a small object which rolled down a ramp that was aligned directly along the subject's midline. Movements of the wrist, index, middle, and thumb were monitored with the WATSMART system. Results showed that movement time and time to peak velocity decreased as target velocity increased. As well, peak velocity of the transport phase increased as target velocity increased; however, at the slowest target speed, peak velocity was actually lower than the peak velocity in the stationary condition. Our results suggest that the movement kinematics of the target strongly influence the movement kinematics of the subject's limb. Target movement however, had no influence on the size of peak aperture or control of the grasp that was a "higher order" control variable. (Supported by NSERC).

553.20

OVERSHOOT IN THREE-DIMENSIONAL POINTING MOVEMENTS. O. Forden, M. Berkholz*, S. Adomovich*, and H. Pinzke. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, Academy of Sciences, Moscow.

Pointing movements were executed by right-handed normal subjects with their eyes closed using their right index finger or a pointer 30 cm in length. Most of the movements were executed from an initial position in which the upper arm was vertical and the elbow strongly flexed, so that the index finger was close to the shoulder joint. A programmed robot arm was used to position 10 targets in 3D space. The movements of the subjects and the robot arm were digitized in 3D with a Watsmart system, and analyzed numerically and graphically. The subject had to begin movement immediately after closing his eyes and was instructed to touch the target on the tip of the robot's arm during a restricted period of time. Since there was no instruction for the subject to come to the target with a zero velocity, overshooting of targets often occurred. The radial "error" was larger when the distance from the shoulder to the target was longer. However, the distance in 3D space between the target and the final position of the tip of the robot's arm was larger than "error" was found to depend upon the target's location. The 3D "error" could be larger when the target was closer, and shorter when the target was more distant. Finally, for one of the subjects, a 5 sec. delay was introduced between the time the subject closed his eyes and the sound signal to move was given. This delay caused no essential decrease in the movement's efficiency. The possible mechanisms of the observed overshoot in pointing movements will be discussed with respect to dynamic regulation of limb posture.
Systematic Directional Errors in Planar Arm Movements
We have previously shown that in targeted arm movements normal human subjects make systematic errors in endpoint-extend extant errors which result from incomplete compensation for variations in the inertial field of the arm. We now report 2 types of systematic errors in movement direction. Subjects moved an arm from a central starting position to 24 equidistant radial targets on a horizontal digitizing table at shoulder level. Target and cursor positions were displayed on a computer screen; the cursor was blanked during movement. Starting hand position was varied in different blocks of trials. Vision of the arm was allowed in some blocks and prevented in others. All subjects showed direction-dependent directional errors manifested as systematic clockwise (CW) errors in certain directions and counterclockwise (CCW) errors in others. These results clustered with extrinsic coordinates of target location. (NS 22715)
Direction-independent errors represent inaccuracies in the specification of movement dynamics, since they vary with limb inertia. Direction-independent errors represent inaccuracies in the specification of joint kinematics as though the nervous system programs joint rotations assuming a midline starting position of the body. Direction-independent errors which are regulated by central commands. The issue of whether kinematic and electromyographic (EMG) patterns. EMG activity was recorded from biceps, triceps, posterior deltoid, and pectoralis major and arm positions were recorded in 3-D with Watsmart. Movements of varying rate to both single- and double-step visual targets were examined. The results further support the hypothesis that horizontal reaching movements are planned in coordinates of the EP of the endpoint. Moreover, the findings suggest that movements of different speeds are achieved by shifting the EP of the endpoint at different rates. The model is used to examine the interaction between the rate of shift of the EP and the level of co-contraction, both of which are regulated by central commands. The issue of whether kinematic and EMG patterns of double-step movement trials can be predicted by the superposition of patterns of single-step movement trials is investigated.

A MODEL OF HUMAN TARGET-DIRECTED HORIZONTAL ARM MOVEMENT J.R. Flanagan, A.G. Feldman and D.J. Ostry. McGill University, Montreal, Canada, Institut de genie biomédical, Université de Montréal, Montreal, Canada
Last year we presented experimental and simulated trajectories of rapid horizontal arm movements directed towards stationary and displaced visual targets. Simulations were generated with a biomechanical model of two-joint planar arm movement. The model, which is based on the equilibrium point hypothesis, assumes that (a) arm movements are planned in coordinates which specify the equilibrium position (EP) of the endpoint and (b) that the EP of the endpoint shifts at a constant rate towards the presently illuminated target. This year the model, which has been extended to include accurate muscle moment arms, is used to predict both kinematic and electromyographic (EMG) patterns. EMG activity was recorded from biceps, triceps, posterior deltoid, and pectoralis major and arm positions were recorded in 3-D with Watsmart. Movements of varying rate to both single- and double-step visual targets were examined. The results further support the hypothesis that horizontal reaching movements are planned in coordinates of the EP of the endpoint. Moreover, the findings suggest that movements of different speeds are achieved by shifting the EP of the endpoint at different rates. The model is used to examine the interaction between the rate of shift of the EP and the level of co-contraction, both of which are regulated by central commands. The issue of whether kinematic and EMG patterns of double-step movement trials can be predicted by the superposition of patterns of single-step movement trials is investigated.

MUSCLE ACTIVATION WAVEFORMS AND ARM MOVEMENTS IN THREE-DIMENSIONAL SPACE.
M. Flanders. Dept. of Physiology, Univ. Minnesota, Minneapolis, MN 55455.
This study related the temporal waveforms of muscle activation to the direction or velocity of the movement of a human arm to targets in three-dimensional space. For the study of direction, targets in different directions were placed in the sagittal plane and the frontal plane, the initial arm position and movement amplitude were constant, and the velocity was moderate (movement time was about 500 ms). For the study of velocity, movement trials were varied between about 200-1000 ms. Statistical techniques were used to quantify temporal patterns of electromyographic (EMG) activity in 7-9 elbow and/or shoulder muscles. These techniques included a best covariance procedure to calculate temporal delays and a principal component analysis to view the shape of basic EMG waveforms.

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The analysis revealed simple neural strategies for generating mechanically complex movements, e.g., basic EMG waveforms are scaled in amplitude and delayed in time to generate movements in different directions.

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554.1
DUAL INHIBITION OF THE CRAYFISH’S CLOSER MUSCLE BY THE STRETCHER AND COMMON INHIBITORS. T.J. Wiens. Dept. of Zoology, Univ. of Manitoba, Winnipeg, Canada R3T 2N2.

A study of the roles of inhibitory motoneurons in the control of the crayfish claw revealed that the stretcher inhibitor (SI) motoneuron, previously thought to inhibit only the stretcher muscle, also innervates many fibers of the closer muscle strongly and consistently; it does not inhibit any further claw muscles. The same applies to the SI’s of the crayfish’s walking legs. The claw’s common inhibitor (CI) likewise routinely inhibits parts of the closer and stretcher muscles and all other cheliped muscles, with one exception: CI input to the claw’s opener muscle could only be demonstrated in some 25% of the claws examined. The CI’s of walking legs were confirmed to innervate all muscles including the opener. SI’s and CI’s unusual distributions may represent adaptations to the closer muscle’s size advantage over the opener. The opener inhibitor (OI) was confirmed to innervate only the opener muscle. SI’s and CI’s soma were identified intracellularly and stained in claw-ganglion preparations. Both somata lie near the midline, CI’s being slightly contralateral and nearer the posterior end of the ganglion. Their form and function suggest homology between the three crayfish limb inhibitors and those of the locust (Watson et al. 1985).

I thank W.J. Heitler, St Andrews, for facilities.

554.2
Spiking activity of the common inhibitory motoneuron in crustacean leg. Synaptic connections with excitatory motor neurons and sensory afferents. M. Beyenagi* and F. Clarac. Laboratoire de Neurosciences Fonctionnelles, CNRS-LNF2, B.P. 71, 13402 Marseille Cedex 9, France.

The motoneuronal command of the leg muscles in crustacean is composed by excitatory motoneurons specific for each muscle and by a common inhibitory motoneuron (CI) which innervates every leg muscle. During rhythmic locomotory behaviors, such as swimming and walking, excitatory motoneurons may rhythmic bursts of spikes patterned to ensure coordinated muscle contractions while the CI motoneuron fires tonically with an increase of firing frequency at the end of the return stroke. Pre- and post-synaptic inhibition of CI at the neuromuscular junction are performed to suppress contraction of the slow muscle fibers and to speed up relaxation of the fast muscle fibers during leg movements. In isolated nervous preparations, central synaptic relations between excitatory and CI motoneurons are few and correspond to weak excitatory monosynaptic and/or weak inhibitory polysynaptic relations. However leg sense organ afferences have strong mono- and polysynaptic connections with the CI motoneuron. These relations suggest a separate central control of the spiking activity of the excitatory and the CI motoneurons and a specific peripheral control by the sense organs during locomotory behaviors.

554.3
INNERVATION OF THE SWIMMERETS OF CRAYFISH: A PAIR OF MODULAR PATTERN-GENERATING CIRCUITS IN EACH ABDOMINAL GANGLION. B. Mullenoy, W.M. Hall, C. M. Shoff, G. Brown-Weninger* and A. Chabot*. Zoology and Neurobiology, Univ. California, Davis CA 95616 USA.

Each swimmer on the abdomen of the crayfish, Pacifastacus leniusculus, is innervated exclusively through one of the paired first segmental nerves, NI, in its segment. Backfills revealed that each NI contains axons of sixty eight swimmer motoneurons, two non-spiking stretch receptors and many other sensory afferents. Cell bodies of these motor neurons were located ipsilateral to their axons, along with accompanying vocal fold tension and length. Network training minimizes the difference between desired and produced vocal fold tension and to speed up relaxation of the fast muscle fibers during leg movements. In isolated nervous preparations, central synaptic relations between excitatory and CI motoneurons are few and correspond to weak excitatory monosynaptic and/or weak inhibitory polysynaptic relations. However leg sense organ afferences have strong mono- and polysynaptic connections with the CI motoneuron. These relations suggest a separate central control of the spiking activity of the excitatory and the CI motoneurons and a specific peripheral control by the sense organs during locomotory behaviors.
554.5

NEURAL POSTURAL REFLEXES IN LOBSTERS DEPEND UPON CENTRAL PROGRAM. S.C. Sukhdeo and C.H. Page. Dept. Biol. Sci. Rutgers University, New Brunswick, NJ 08903-0750. Stretch stimulation of the abdominal muscle receptor organ (MRO) of the lobster, Homarus americanus, initiated a spike discharge of its tonic sensory neuron (SRL). This sensory response evoked a series of reflex responses in the afferents of the superficial extensor and flexor muscles of the system. The type of motor response varied according to whether a flexion or extension pattern of spontaneous activity was generated by the postural afferents.

During spontaneous centrally-initiated flexion motor programs, SRL discharge elicited an assistance response which included excitation of a medium size flexor excitator (E3) and the peripheral extensor inhibitor (E5), and inhibition of at least one extensor excitator. While during spontaneous centrally-initiated extension motor programs, SRL spiking triggered a resistance response that included excitation of the extensor excitators and the flexor peripheral inhibitor (E5) only, F3 and E5 spontaneous activities were unchanged. Thus reflex reversal occurs between assistance (flexion) and resistance (extension) reflexes.

554.7

BIOTIN-STAINING IN THE LOBSTER GIANT-NEURON SYSTEM. H. Schneider, P.M. Ma & E.A. Kravitz. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115. The lateral (LG) and medial giant (MG) neurons in the lobster nervous system play well known roles in escape behavior. Anatomically, these cells are characterized by large-diameter axons extending through the entire length of the ventral nerve cord. We found that these cells also are characterized by a high endogenous content of the vitamin biotin. Due to specific and high affinity binding of biotin to avidin, labeled avidin was used to visualize biotin in whole mounts of the giant neuron axons using fluorescence microscopy.

We are currently using this simulation to evaluate the circuit components responsible for the long lasting firing of the LFS siphon motor neurons which occurs in response to siphon stimulation. Our initial finding is that the slow component of interneuron synaptic transmission contributes to the maintenance of the slow firing of the LFS siphon motor neurons which is governed by the pedal ganglion (PedG) involved in locomotion. Cobalt backfills of the pedal nerve labeled 11 somata at the medial lobe and numerous others at the anterior pole of the PedG. Many of these neurons are known to have a serotonin-activated Ca2+-dependent Na+ current (I_{Ca2+Na+}); Huang & Gillette, Soc. Neurosci. Abs. 13:459, 1987). Single channel analysis of I_{Ca2+Na+} yielded an opening time constant of 2.27 ± 0.12 ms with a conductance of 40 pS. It was surprising to find that many of the neurons also exhibited serotonin immunoreactivity. Self-regulation of these serotonergic neurons in the PedG may contribute to the prolongation of the slow firing duration in the locomotory activity of the slug.

554.6

MORPHOLOGY OF GIANT AXONS AND THEIR TARGET NEURONS IN LOBSTERS. P.M. Ma. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115. In lobsters, serotonin and octopamine are neurohormones that modulate many behaviors, including escape tail-flips mediated by the medial (MG) and lateral (LG) giant axons in the ventral nerve cord. For example, serotonin decreases the excitability of giant axons by modulating their primary afferent inputs. To explore the possibilities that amines also modulate escape behavior on the output side, studies were performed to examine the structure of giant axons and to locate and identify their target neurons. The giant axons of larval and juvenile lobsters were dye-injected and the branching patterns of the axons within the ganglia were analyzed. LG and MG axons were tentatively considered to be electrically coupled. MG axon originate in the brain and project, with few branches, to the caudal ganglion. Coupling between the pair of MG axons occurs in both the brain and the caudal ganglion. LG axons are a synaptotagmin with contributions from neurons in the thoracic and abdominal ganglion. These axons extend through the entire nervous system are correlated with the modulated firing frequencies.
554.11 CHEMOSENSORY RESPONSE TO METAMORPHIC INDUCER ACTIVATES A CENTRAL PATTERN GENERATOR IN A LARVAL MOLLUSC. E.M. Leise and M.G. Hadley. Biomedical Research Center, Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI 96813.

Planktonic larvae of the sea slug Phestilla sibogae settle and metamorphose in response to a peptide (300 dalton), water-soluble organic molecule produced by their adult food source, the coral Porites compressa. Sensory receptor neurons in the larval epidermis are probably responsible for recognizing the inducer compound. We hypothesize that excitation of the putative chemoreceptor neurons triggers metamorphosis by activating control circuits in the larval CNS. The events of metamorphosis include relatively rapid phenomena, such as loss of the ciliated swimming organ and detachment of the larva from its shell, as well as sequential tissue rearrangements and cell movements. To test our assumptions we are studying the responses of the larval NS to the metamorphic inducer.

Experiments are conducted on 10 day-old metamorphically competent larvae. Extracellular and intracellular recordings are made in vitro from unanesthetized, disheled and restrained larvae or from isolated larval heads in vitro. The tissue is bathed in filtered seawater (SW), SW containing the inducer compound (I-SW), or SW conditioned by exposure to non-inducing I-SW, or seawater containing the larval swimming organ. One to 2 mins after addition of I-SW, the frequency and duration of compound spikes indicative of velar cirrall arrest change. Spontaneous bursts of action potentials from smaller units become rhythmic (~0.5 Hz) after the preparation is exposed to the inducer for 1 to 2 hrs. Experiments on larvae with only the visceral hump removed suggest that the rhythmic bursts are associated with the stereotypical posture taken by larvae initiating metamorphosis.

554.13 CHARACTERIZATION OF A FEEDING NEURON CONTAINING THE NEUROPEPTIDE APGWα IN THE POND SNAIL, Lymnaea stagnalis. RP. Croll1, CR. McCrohan2*

THE NEUROPEPTIDE APGWα IN THE POND SNAIL, Lymnaea stagnalis. RP Croll1, CR McCrohan2*

LYMNAEA STAGNALIS. RP Croll1, ÇR Mccrohan 2*

THE NEUROPEPTIDE APGWα IN THE POND SNAIL, Lymnaea stagnalis. RP Croll1, CR McCrohan2*

During submaximal contractions (Fuglevand et al., 1985), the surface EMG and abduction force of first dorsal increase in parallel. Surface EMG and abduction force of first dorsal in vivo. The tissue is bathed in filtered seawater (SW), SW containing the inducer compound (I-SW), or SW conditioned by exposure to non-inducing I-SW, or seawater containing the larval swimming organ. One to 2 mins after addition of I-SW, the frequency and duration of compound spikes indicative of velar cirrall arrest change. Spontaneous bursts of action potentials from smaller units become rhythmic (~0.5 Hz) after the preparation is exposed to the inducer for 1 to 2 hrs. Experiments on larvae with only the visceral hump removed suggest that the rhythmic bursts are associated with the stereotypical posture taken by larvae initiating metamorphosis.

554.11 INABILITY OF HUMANS TO FULLY ACTIVATE MUSCLE DURING SUSTAINED CONTRACTIONS AT DIFFERENT FORCE LEVELS. A.J. Endleyard, K.M. Zackowski1, K.A. Haert1, and R.M. Emoea. Dept. Exercise & Sport Sciences and Physiology, Univ. Arizona, Tucson, AZ 85721

During a constant-force submaximal contraction, there is a gradual decline in the force capacity of active motor units. The nervous system, therefore, can increase motor unit recruitment and discharge rate in order to maintain the desired force. The purpose of this study was to examine changes in neural drive to muscle, as reflected in the surface EMG, during sustained contractions. Subjects (n = 32) were instructed to sustain an isometric abduction of the index finger to exhaustion at one of three force levels: 20%, 35%, or 65% of maximum voluntary contraction (MVC) force. Surface EMG and abduction force of first dorsal in vivo. The tissue is bathed in filtered seawater (SW), SW containing the inducer compound (I-SW), or SW conditioned by exposure to non-inducing I-SW, or seawater containing the larval swimming organ. One to 2 mins after addition of I-SW, the frequency and duration of compound spikes indicative of velar cirrall arrest change. Spontaneous bursts of action potentials from smaller units become rhythmic (~0.5 Hz) after the preparation is exposed to the inducer for 1 to 2 hrs. Experiments on larvae with only the visceral hump removed suggest that the rhythmic bursts are associated with the stereotypical posture taken by larvae initiating metamorphosis.

554.12 CORRELATIONS OF SPECIFIC NEURAL PATTERNS AND BUCAL MUSCLE CONTRACTIONS WITH DYNAMIC STAGES OF FEEDING CYCLE. S. Krumholtz, B.C. Arnett1, and A.D. Murphy. Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60680

Rhythmic feeding behavior in Helisoma is characterized by dynamic stages of protrusion, retraction, and hyper-retraction of the feeding apparatus. This behavior is generated by a buccal pattern generator composed of three neuronal subunits (S1, S2, and S3). Each subunit is an independent oscillator that can be temporally linked to either one or two of the other subunits to produce a variety of motor patterns. The neuromodulators serotonin, dopamine, and GABA can reliably phase-lock the subunits to evoke particular neural patterns. In order to correlate these resulting neural patterns with consequent behaviors specific for each neuromodulator, I have simultaneously videotaped movements of the buccal mass and recorded intracellularly from identified buccal motoneurons that provide a monitor of the functional state of the pattern generator. The results are consistent with our hypotheses that: S1 activity mediates the protrusion of the radula and odontophore cartilage and flattening of the buccal mass; S2 activity mediates retraction of these oral structures; and S3 activity results in the hyper-retraction of these structures. Supported by NIH R01 NS26145.

This study investigated the age associated loss of motor units in the biceps brachii-brachialis region and the resultant effect on force production.

The numbers of functioning motor units was estimated from the passive twitch preceding the spike-triggered averaging technique (Brown et al, Muscle & Nerve, 11, 423-432, 1989) in 18 active older adults (age 68 +/- 5 yrs) and 12 active younger adults (29 +/- 5 yrs). The peak to peak amplitude of the compound muscle action potential (M-wave) was significantly lower in older subjects as compared to controls (p<.001) and the surface recorded single motor unit potential was significantly greater (p<.05). Therefore, as expected, older subjects had significantly fewer motor units (195 +/- 85) than controls (352 +/- 81) (p<.001). The electrically evoked isometric twitch contraction did not differ significantly between old and young subjects whereas the maximal voluntary contraction was significantly lower in the older subjects.

555.5 ELECTROMYOGRAPHIC (EMG) AND RANGE OF MOTION (ROM) COMPARISONS BETWEEN STATIC STRETCHING AND PROPRIOPETITIVE NEUROMUSCULAR FACILITATION (PNF) STRETCHING TECHNIQUES IN HUMANS. B.R. Emery and S. Overholt*, Human Performance Laboratory, Rice University, Houston, TX 77251.

Static stretching and two common PNF techniques were compared for hip flexion ROM and the phasic and quantitative muscle activity of stretched femoral muscles. The stretching techniques for each of 15 subjects were: static (SS), contract-relax (CR), and contract-relax-reversal (CRR). Hip flexion ROM was measured before and after stretching. EMG recording was from the subject's rectus femoris and biceps femoris muscles (pre- and post-stretching). Unlike most previous studies, we precluded electrode "cross-talk" between antagonistic muscles by establishing independence of activity of each muscle before testing. The EMG signals were integrated and peak spectral density was determined from fast Fourier transform (FFT) of each trial. All three stretching techniques yielded significant gain in hip flexion ROM compared with the initial condition. PHSS, CRSS, and CRRS = 85°, 82°, and 85°, with no significant differences between stretching techniques. All stretching conditions only one subject demonstrated biceps femoris activity during stretching. CRR condition: EMG = 90.6% of control, peak FFT = 67 Hz. Rectus femoris activity during the stretching reached in average EMG of subjects of 25.8% and FFT = 50.4 Hz. Although the PNF methods resulted in the greatest gain in ROM they were not significantly different from the SS method. While controlling for cross-talk between electrodes of antagonist muscles, co-contraction between antagonist muscles during the reversal method was observed, but it was uncommon, contrary to results of previous studies.


The length-tension relations of nine different muscles in the hind limb of the cat was determined with special emphasis on possible differences that may be function and architecture related. Electrical stimulation at tetanic rates was applied to the sciatic nerve while monitoring isometric force of the muscle by either stimulating it directly or indirectly via the phrenic nerve at frequencies ranging from 10-100 Hz for a brief period of 1 second. We found that, at a given frequency, the adult muscle generated the force with less loss of force via the phrenic nerve. As expected, older subjects demonstrated less force when stimulated via the phrenic nerve. Older subjects were also more sensitive to the frequency of stimulation. In addition, we found that, for a given frequency, the adult muscle generated the force with less loss of force via the phrenic nerve than when stimulated directly at all frequencies used. In addition, at frequencies > 40 Hz, force generation from indirect stimulation was greater than that generated from direct stimulation. We conclude that the newborn and young adult muscle has less tendency for active motor units to discharge synchronously, and the reluctance to activate multiple motor units concurrently active units. These findings are consistent with previous studies showing that the newborn and young adult muscle has less tendency for active motor units to discharge synchronously, and the reluctance to activate multiple motor units.


Spindle discharges during muscle contraction (loading response; LR) and/or relaxation were investigated in the soleus of anaesthetized cats. Single LA spikes were recorded in dorsal roots L7-S1 filaments. Spindle location was found by pressing the muscle with von Frey hairs (12.5 mg). Perpendicular muscle vibration (250 Hz, variable intensity) was applied to soleus muscle. Maximal sensitivity was restricted to a single spot. In fibers showing LR to nerve stimulation were located near either tendon or both. Maximal LR responses (UR) were located at the medial third of the muscle. Contraction of discrete muscle bundles were produced by direct stimulation. UR and relaxation firing (RF) were found stimulating on the receptor but stimulating 2-4 cm away in the longitudinal direction, LR appeared and RF decreased or disappeared. Lateral stimulation only gave UR. Thus, spindles are coupled both in-series and in-parallel with extrafusal fibers.
555.9
BIOMECHANICAL ACTIONS OF MULTARTICULAR MUSCLES IN THE CAT NECK. W.S. Selles*, D.B. Thomson, and E.J.R. Richmond. MRC Group in Sensory-Motor Physiology, Queen’s University, Kingston, Ont. K7L 3N6 CANADA.

Our goal is to describe biomechanical features of head movement in the cat. In an attempt to determine centres of rotation of muscles in the sagittal plane we have studied cat neck movement. It is our hypothesis that the proximal and distal ends of a muscle may function independently of each other in determining movements of the head and neck. We have determined that the cat neck is a complex system of interacting muscles that can produce a wide range of movements with the head and neck.

555.10

In glycogen-depletion experiments we have shown that the proximally- and distally-directed nerve branches entering anterior sartorius supply asymmetrically distributed motor-unit territories. These territories are organized partly in parallel, partly in series. Force-length relations of anterior sartorius have now been examined by stimulating either the parent nerve bundle or the individual nerve branches supplying the muscle. It was suggested that the resulting force-length curves were not simply a scaled-down version of the force-length curve produced by stimulating the parent nerve bundle; at long whole-muscle lengths, force of the partially activated muscle was enhanced whereas at short whole-muscle lengths, it was diminished. The force-length relations suggested that a complex interaction of both passive and active elements contributed to total force. It remains to be confirmed whether selective recruitment occurs amongst the different subpopulations of motor units, as suggested by reflex studies of Sherrington (1910).

555.11

The success of functional electrical stimulation to reanimate paralyzed muscles will depend on the ability to position stimulating electrodes appropriately within target muscles. Long muscles such as gracilla and sartorius pose a particular challenge because of their complex architectural arrangement. The short, tapering, innervation pattern of these muscles may or may not be concurrently active. If individual muscle units are not distributed along the length of the muscle, mechanical instabilities may arise. These studies, single motor-units were glycoen-depleted by stimuli delivered either intracellularly to motoneurons or extracellularly to single ventral-root axons. In all motor units studied so far (n=8), fibers were distributed asymmetrically, with a relatively high density at one end or in the middle of the muscle, decreasing or disappearing over the remaining length of the muscle. The potential contribution of each muscle to the production of a given movement depends on the instantaneous magnitude and direction of its moment arm and the distribution of motion between its muscle fibers and series-elastic connective tissues. As is typical of many joints, the ankle has multiple muscles acting upon multiple degrees of freedom. Many of the ankle muscles have very short moment arms combined with highly pinnae architectures, which makes direct measurement of these critical parameters difficult and unreliable. We have employed sonomicrometry to measure small increments of motion between bone, tendon and muscle fascicles when the ankle is clamped in various positions of extension/flexion and inversion/eversion during both passive and active conditions. The resultant muscle paths for some long muscles actually passed through the cervical column in some postures and thus required sonomicrometric analyses.

555.12

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555.13
A COMPUTER-MODELING ENVIRONMENT FOR THE ANALYSIS OF KINESELOLOGY, BIOMECHANICS AND SENSORIMOTOR CONTROL IN MULTARTICULATED LIMBS. G.E. Loeb, Z. Hu, P. Chapey and W.S. Leutscher*. Bio-Medical Engineering Unit, Queen’s University, Kingston, Ont. K7L 3N6 CANADA and Dept. of Electrical Engineering, University of Maryland, College Park, MD 20743 USA.

Body movement results from the result of a complex interplay between the intrinsic mechanical properties of musculoskeletal systems and their dynamic modulation by the nervous system. For all but the simplest and most constrained systems, an appreciation of their relationships requires quantitative integration and presentation of large amounts of diverse data regarding morphometry, kinematics and dynamics. We have developed an interactive environment that we call the Macintosh II personal workstation that supports the following functions: Morphometric spreadsheet - generalized system for describing and dimensioning multiple anatomical descriptions; Analog and video data acquisition - timecode-synchronized spreadsheet files of EMG, transducer and joint position data; KINESIOLOGY, BIOMECHANICS AND SENSORIMOTOR CONTROL IN MULTARTICULATED LIMBS.
556.1

EFFECTS OF DORSAL NEOCORTICAL STIMULATION ON PERFORANT PATH EVOKED FIELD POTENTIALS IN THE DENTATE GYRUS OF THE RAT. D. L. Korol, J. W. Leonard, B. L. McNaughton, A. C. Barnes. Department of Psychology and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Modulation of neural excitability in the hippocampus results from activation of cortico-hippocampal afferents. Precipitation of the medial septal and supramammillary nuclei facilitates the population spike (PS) of dentate granule cell field potentials evoked by perforant path (PP) stimulation, probably by suppressing inhibitory interneurons (Mizumori et al., 1989). To address the question of how cortical affections other than the primary input from entorhinal cortex affect hippocampal dynamics, we examined the effects of the activation of PF zones of various neocortical areas on PP-evoked EPSPs and population spikes (PSs) in anesthetized rats. Substantial facilitation of the population spike, with little or no change in the field EPSP, can be evoked by stimulation of some cortical sites, whereas inhibitory effects are observed at other sites. Preliminary mapping studies suggest a clustering of excitatory fields in frontal (motor) and parietal (sensory) (HL) areas; however, the effects are clearly at least disynaptic, and state-dependent. More data will be required to determine whether this clustering is consistent. Cortical activation alone often elicited field potentials with latencies varying from 3-16 msec that were correlated with the incidence of facilitation; however, the cortical potential failed to exhibit polarity reversal in the molecular layer, whereas the simultaneously evoked PP potential did reverse polarity. This suggests that the cortically evoked potential is not generated in dentate gyrus.

Supported by NS45331, AG05040 and MH42649.

556.4


The transcription factor zif 268 can be rapidly activated in granule cells in the hippocampus by patterned electrical stimulation that induce LTE in both anesthetized and chronically-prepared young rats. Like LTE, zif 268 induction is dependent on NMDA receptor activation. In old rats, mechanisms of LTE induction appear to be normal, but the maintenance of LTE is deficient. Accordingly, it was of interest to determine whether mechanisms of zif 268 activation are defective in old rats. As a first step we examined 4 groups of Fischer 344 rats were tested: young (N=5) and aged (N=7) controls and an aged group (N=5) administered Acetyl-l-carnitine (Acetyl-l-carnitine (AC), an ester of the endogenous compound carnitine appears to improve, for example, the morphological changes that normally occur in the hippocampus of aged rats (Ramacci et al., 1989). To address the question of how cortical affections other than the primary input from entorhinal cortex affect hippocampal dynamics, we examined the effects of the activation of PF zones of various neocortical areas on PP-evoked EPSPs and population spikes (PSs) in anesthetized rats. Substantial facilitation of the population spike, with little or no change in the field EPSP, can be evoked by stimulation of some cortical sites, whereas inhibitory effects are observed at other sites. Preliminary mapping studies suggest a clustering of excitatory fields in frontal (motor) and parietal (sensory) (HL) areas; however, the effects are clearly at least disynaptic, and state-dependent. More data will be required to determine whether this clustering is consistent. Cortical activation alone often elicited field potentials with latencies varying from 3-16 msec that were correlated with the incidence of facilitation; however, the cortical potential failed to exhibit polarity reversal in the molecular layer, whereas the simultaneously evoked PP potential did reverse polarity. This suggests that the cortically evoked potential is not generated in dentate gyrus.

Supported by Sigma Tau Corp.
556.5

AGE-RELATED DECREASE IN CA1 EPSP TO FIBER POTENTIAL RATIO. G. Rao, C. A. Barnes, T. C. Foster and B. L. McNaughton. Dept. of Neurosci. and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

We previously reported an age-related decrease in sensitivity to extracellularly applied AMPA in hippocampal slice CA1, based on the ability of this glutamate synaptic receptor agonist to attenuate in situ evoked field EPSPs, as well as on a reduction of extracellular field potentials induced by local application of AMPA in stratum radiatum (Soc. Neurosci. Abst., 16: 776, 1990). If a decrease in post-synaptic sensitivity underlies this observation, it should also be manifested in a decrease in the amplitude ratios of the CA1 EPSP and fiber potential components.

Hippocampal slices were prepared from F-344 male rats at ages 6 and 27 mos (n = 7 rats per group). A recording pipette and a stimulating electrode were positioned in stratum radiatum and five extracellularly evoked potential responses were collected at four intensities (50, 100, 200 and 400 μA). The amplitude of the initial phase of the EPSP and the area of the fiber potentials were subsequently analyzed. At all four intensities, the EPSP to fiber potential amplitude ratios were smaller in the old animals. The decline was significant (p<.005) at the 100 and 200 μA intensities. This result is consistent with the AMPA results described above.

Experiments employing minimal stimulation to activate single fiber and quantal analysis to estimate mean quantal parameters are in progress to determine whether the reduced AMPA responses and EPSP to fiber potential ratios, are accompanied by a reduced apparent quantal size. Supported by AG03376.

556.6


We reported that a 30% increase in mean EPSP amplitude during long-term synaptic enhancement (LTE) is due to an increase in quantal size (q) in the absence of changes in transmitter release (Foster, McNaughton, Hippocampus, 1991). Malinow and Tsien (Nature, 1990) reported a substantial EPSP increase (200%) due to a large increase in m and a small increase in q. We have attempted to determine the factors responsible for the disparate results, including: 1) immature (3-5 wk) rats, 2) reduced temperature (22°C), 3) elevated Ca++, 4) method for LTE induction (pairing 40 pulses, 2 Hz, with postsynaptic depolarization), and 5) slow stimulation rates. No single factor accounts for the high quantal size and/or large increases in m as reported by Malinow and Tsien, which results in larger m at 2°C. However, the lack of increase in q in the present study may be due to using a lower stimulation rate. Whereas, in our studies, there was no average change in m, the magnitude and direction of changes in m following LTE stimulation were negatively correlated with baseline m. Changes in m were mainly due to changes in p. Since p cannot be, a floor effect may result from initially low values. The initial value of m, calculated from the mean number of transmission failures for 5 cells reported by Malinow and Tsien, was substantially lower than in most other studies of these synapses. Thus the discrepancy between the two studies may be related to differences in the aging protocol; however, we have no explanation for why Malinow & Tsien did not observe increased m after stimulation in the absence of depolarization. Supported by NS08580 to TCF and NS20331 to BLM.

556.7

ADDITIVE RELATIONSHIP BETWEEN PERFORANT PATH LTE/LTP AND STEM SUGGESTS INVOLVEMENT OF DIFFERENT SYNAPTIC POPULATIONS. B.L. McNaughton, C.A. Erickson, C.A. Barnes GD. Stevenson. Dept. Psych. and ARL Div. of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Exploratory behavior leads to a short-term (15-30 min) elevation of synaptic responsiveness blocked in the fascia dentata by electrical stimulation of the perforant path (Sharp et al., Psychobiol., 12: 257-269,1989; Green et al., J. Neurosci., 10: 1455-1471, 1990). Although not permanent, this short-term exploratory generated LTP (LTE) that were correlated with a decreased loco motor component of exploration using a motorized treadmill. Unlike stimulus induced long-term enhancement (LTE), STEM is due to a change in synaptic strength that appears to be stable over days. The decline is significant (p<.005) at the 100 and 200 μA intensities. This result is consistent with the AMPA results described above.

To address the question of whether STEM and LTE share a common mechanism of expression we studied the manner in which the two phenomena interact. Possible interactions are occlusion, suggesting a common memory trace, or multiplicative relationship, possibly due to one pre- and one postsynaptic effect, and an additive relationship, suggesting independent sites of expression. The absolute, STEM-related EPSP growth (mean 43%) was virtually identical (slope = 1.03) before and after LTE induction (mean 42%), indicating a purely additive relationship. Moreover, whereas LTE was characterized by an increase in EPSP slope, STEM involved an apparent reduction in EPSP onset latency. These data suggest that STEM and LTE may occur at different synapses, and, as suggested by Green et al. 1990 on other grounds, that STEM could represent a rather large growth at a small proportion of synapses. Supported by O.M.R. & AC03376.

556.8

40-HZ "FLUTTERING" OSCILLATIONS IN THE RAT HIPPOCAMPUS. W.E. Skaggs, B.L. McNaughton and C.A. Barnes. Deps. Neurosci. and Psych. and ARL Div. of Neurosci. Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724. Email: bill@bimsa.arizona.edu

The hippocampus, because of its large number of cells and laminar architecture, is capable of generating strong extracellular electric fields. In addition to the well studied 6-8 Hz "delta" rhythm, we find that it also gives rise to higher frequency oscillations. These can be heard on an audio monitor as a distinctive "fluttering" sound when a recording electrode passes through the molecular layer of CA1 and approaches or enters the CA1-CA3 border cell layer (the audible component is transient, and appears to be generated by local neuronal injury discharge, which occurs in phase with the underlying EEG oscillations). A series of studies were conducted to characterize these higher frequency EEG oscillations. They are highest in amplitude (about 0.2 μV) in the upper molecular layer of the fascia dentata, and are highly correlated even between the two hemispheres. They appear in anesthetized as well as waking animals, but in waking animals they fluctuate in frequency from 40 to 70 Hz, while under general anesthesia they are slower (35-45 Hz) and more regular. They increase in amplitude when an animal is actively exploring its environment. These oscillations appear to be generated both in CA1 and in the fascia dentata. It is hypothesized that the oscillations are caused by "ringing" of a feedforward excitation -- feedback inhibition network of glutamatergic granule cells or pyramidal cells and GABAergic basket cells, and that the timing of the oscillation is set by the decay time constant of GABA-a receptor channels. Supported by NIMH grant MH46683.

Leonard (Psychol. Rev., 99, 1992) noted that, in experiments which animals had extensive experience with the environment beyond the test apparatus, substantially fewer hippocampal pyramidal cells had discernable "place fields" on the apparatus. Leonard also noted that groups of animals with extramaze experience sometimes had apparent place fields as the animal was carried passively through the environment. These observations, however, are not the number of possible alternative explanations, due to confounding variables such as task demands and behavior. In this experiment, we queried whether size of the visually accessible environment would affect the distribution of place specific firing within a small circular apparatus. Two identical 76 cm diameter platforms were used, one with a high cylindrical wall that restricted the animal's access to distal visual stimuli. The other cylinder was transparent. The opaque wall was covered with complex patterns. In the transparent condition, similar patterns were mounted on the room walls. The same cells were tested in both conditions. Behavior (random search for food pellets) was the same in both conditions. Mean firing rates in both conditions were highly correlated (.98) and not different; however, the presence of clear place fields differed substantially. In the distal condition, 4/49 cells had high spatial selectivity. In the proximal-only case, 15/49 cells were highly selective. A paired t-test on the spatial selectivity scores was significant (p < .0001). We hypothesize that, even without direct physical experience, place fields spread out to occupy the known space. Supported by NS02331

556.12 AUDITORY AND HIPPOCAMPAL NEURONAL ACTIVITIES DURING PERFORMANCE OF AUDITORY WORKING AND REFERENCE MEMORY TASKS IN THE RAT. Y. Sakurai. Dept. Psychology, Toyoa Med. & Pharma. Univ., Sugitani, Toyoa 930-01, Japan. The auditory cortex (AC) and medial geniculate body (MGB) are related to auditory processing and memory. To explore the role of hippocampus (CA1 and CA3) in auditory tasks, we examined correlations between hippocampal and auditory memory tasks in a single situation. The working memory task was continuous nonmatching-to-sample with two groups of rats: (Go) if the tone for the current trial was different than the tone for the preceding trial. The reference memory task was continuous discrimination. The rats made a Go response if the tone for the current trial was high. The apparatus, tone stimuli and the sequence for continuous presentation of the tones were identical between the working and reference memory tasks. Some of the units had mnemonic correlates during tone presentations or delay periods in one of the tasks. These correlations were consistent across groups of rats and between behaviors. We conclude that the hippocampus exerts a significant influence on auditory memory by means of mnemonic correlates between neurons of the hippocampus and auditory cortex. The presence of clear place fields differed substantially. In the proximal-only case, 15/49 cells were highly selective. A paired t-test on the spatial selectivity scores was significant (p < .0001). We hypothesize that, even without direct physical experience, place fields spread out to occupy the known space. Supported by NS02331

556.13 EFFECTS OF THE NMDA ANTAGONIST, MK-801, ON SHIFTS IN THE EPSP TO SPIKE RELATIONSHIP FOLLOWING LONG-TERM POTENTIATION OR SHORT-TERM EXPLORATORY MODULATION OF DENTATE GYRUS EVOKED POTENTIALS. R. D. Croll and E. Rostock. Dept. of Psychology, Queens College-CUNY, Flushing, NY 11367.

The EPSP to spike (E-S) relationship occurs following induction of long-term potentiation (LTP) or short-term exploratory modulation (STEM) of dentate gyrus evoked potentials. In the dentate hulus, the E-S relationship shifts observed following induction of LTP or STEM. In addition, effects of 5 mg/kg sc MK-801 (injected 30 min prior) on these effects were examined. Following LTP induction, a significant leftward shift of the E-S relationship was observed. This shift was blocked and reversed to a rightward shift following MK-801 administration. Animals receiving no LTP following exploratory modulation showed no shift in the E-S relationship. During STEM, a significant rightward shift of the E-S relationship was observed. This effect persisted for at least 20 min. This rightward shift was significantly reduced by MK-801 administration. These findings suggest a role of the NMDA receptor in the E-S relationship shifts observed following induction of either LTP or STEM.

556.14 INDUCTION OF HIPPOCAMPAL PKC EXPRESSION BY SPATIAL DISCRIMINATION LEARNING IS ABOLISHED BY AMYGDALOID KINDLING. H. A. Kindt, D. M. A. Eikelboom, E. Van der Zee*, H. J. A. Beld and E. A. van der Zee*, H. J. A. Beld-­... Dept. of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA HAREN, The Netherlands. (Supported by the Netherlands Organisation for Scientific Research.)

Recent evidence suggests the involvement of protein kinase C (PKC) in the molecular mechanisms by which the hippocampus exerts its influence on information processing. Normal hippocampal function is of importance for these processes. In the following experiment we investigated the contribution of PKC to spatial discrimination performance. We were interested whether abnormal functional activity induced by kindling and the correlated changes appearing in the neural substrate. In the proximal-only case, 15/49 cells were highly selective. A paired t-test on the spatial selectivity scores was significant (p < .0001). We hypothesize that, even without direct physical experience, place fields spread out to occupy the known space. Supported by NS02331


Protein kinase C (PKC) is a key enzyme for signal transduction and various neuronal plasticity mechanisms. Changes in hippocampal PKC content have been reported after non-invasive conditioning tests. Here we examined the expression of PKC in rats after different types of learning tasks: Spatial Orientation (SO), Active (AA) and Passive (PA) learning. We observed that PKC expression was directed toward the septum with complex patterns. In the transparent condition, similar patterns were mounted on the room walls. The same cells were tested in both conditions. Behavior (random search for food pellets) was the same in both conditions. Mean firing rates in both conditions were highly correlated (.98) and not different; however, the presence of clear place fields differed substantially. In the distal condition, 4/49 cells had high spatial selectivity. In the proximal-only case, 15/49 cells were highly selective. A paired t-test on the spatial selectivity scores was significant (p < .0001). We hypothesize that, even without direct physical experience, place fields spread out to occupy the known space. Supported by NS02331


It has been documented that preexposure to conditioned stimulus delays the acquisition of classically conditioned responses (Lubow, 1989; Solomon & Moore, 1975). Since hippocampal neural activity precedes and models the behavioral conditioned response, we hypothesized that preexposure to both CS and UCS would delay the acquisition of behavioral and hippocampal conditioned responses. Bilateral recordings were made during jaw movements during fear conditioning (TDD). Group TDD animals received 8 days of TDD training while Group UNP animals received 5 days of unpaired presentations of the same stimuli and then 8 days of TDD training. In the TDD task, one tone (CS+ 8KHz) signalled a saccharin solution; another tone (CS- 18KHz) signalled the UCS. The UNP group was presented a random-sequence of two tones and saccharin and therefore received 240 preexposures of each stimulus before TDD training started. While we did not find a significant difference in behavioral acquisition rates during TDD training, our preliminary neural results for the UNP group indicate an inhibition of firing just after tone onset in early trials that disappears by the end of the first day of conditioning.
OPTICAL MEASUREMENT OF NEURONAL ACTIVITIES OF HIPPOCAMPUS IN VIVO. T. INUMA and G. MATSUMOTO. Electrotechnical Lab., Tsukuba, Ibaraki 305, Japan.

It has been believed that the hippocampus is composed of multiple "lamella units", each of which includes basically similar excitatory pathway. However, we found the heterogeneity of neuro-circuit in each "lamella unit" through in vitro experiments using hippocampal slice preparations.

To reveal the real information processing in hippocampus, it is necessary to obtain the neuronal activities from the multisites of an intact hippocampus. For the purpose we tried the optical recording of neuronal activities from the surface of guinea pig hippocampus in vivo. The hippocampal surface was exposed by removing a part of cortex. The neuronal activities were recorded from the alvear surface of the hippocampus stained with a voltage-sensitive dye RH 795. When a stimulus was applied to ipsilateral hippocampus, neuronal activities were obtained from a part of hippocampus as the fluorescence change of about 0.3%.

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557.1

Rats were injected intracerebroventricularly with 5,7-dihydroxytryptamine (150 μg/20 μl) to destroy serotonin-containing neurons. Administration of 8-OH-DPAT (5 μg/1 μl) in the CA1 region of the dorsal hippocampus, 10 min before the training trial in a passive avoidance task, significantly decreased retention latencies 24 h later. No antidepressant or anxiolytic-like effects were found with intrahippocampal 8-OH-DPAT. The results suggest that activation of 5-HT1A receptors in the hippocampus interferes with mechanisms involved in learning and memory.

557.2
THE EFFECTS OF 8-HYDROXY-2-(CI-H-PROPYLAMINO)-TETRAHYDRAZINE (8-OH-DPAT) ON DELAYED MATCHING-TO-SAMPLE PERFORMANCE IN RATS. D. Shurtleff and S.R. Ahlers. Naval Medical Research Institute, Bethesda, MD 20889.

The hippocampus, an area important in working memory, contains a high density of serotonin 5-HT receptors. Acute administration of the 5-HT1B agonist 8-OH-DPAT reduced matching accuracy in a delayed matching-to-sample (DMTS) task. In the present study the effects of 8-OH-DPAT on working memory were evaluated using a delayed matching-to-sample (DMTS) task. Rats were given saline or 0.1, 0.25, or 0.5 mg/kg 8-OH-DPAT before the training trial or before the retention test. 8-OH-DPAT impaired DMTS performance, suggesting the involvement of the 5-HT1B receptor subtype in working memory.

557.3
THE SEROTONIN 5HT1A AGONIST TRIFLUOROMETHYLPHENYL-L-PHENYLALANINE (TPFPF) IMPAIRS PERFORMANCE ON A DELAYED MATCHING-TO-SAMPLE TASK IN RATS. S.T. Ahlers1, J.R. Thomas1, and J.L. Davis2. 1 Naval Medical Research Institute, Bethesda, MD 20889.

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) may play an important role in learning and memory. It has also been suggested that 5-HT abnormalities may mediate some aspects of the cognitive disorders associated with Korsakoff syndrome and Alzheimer's Disease. We evaluated the effect of intracisternally applied serotoninergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) on learning and memory in rodents. Three day old rats were treated subcutaneously 5 min after the training trial and 5,7-DHT (50 μg/pup) and pargyline (40 mg/kg, ip) followed by 5,7-DHT (50 μg/pup) and returned to the dam for a month. At 53 days of age the tryptophan hydroxylase (TPH) activity was assayed radioenzymatically with "C"-tryptophan in the dorsal raphé nuclei, caudal brainstem and hypothalamus in some animals. 5,7-DHT treated animals demonstrated marked reduction (90%) in TPH activity compared to matched controls. At 75 days of age, rats were tested on a learning set problem in the Morris water maze for 5 days followed by 30 days of testing in a 12-arm radial maze with 8 of the 12 arms baited. In the Morris water maze, the latency to locate the hidden platform and the swim path length did not significantly differ for 5,7-DHT treated and control rats (E<1.0). Similarly, 5,7-DHT treated rats did not enter arms more frequently than control rats (working errors), or enter unbaited arms more frequently (reference errors) (E<1.0).

These results suggest that 5-HT does not play a critical role in processing of spatial information.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
METHYLEDIOXYAMPHETAMINE: EFFECTS ON BEHAVIOR AND SEROTONIN. A.G. Romano, J.A. Harvey, J.E. McElroy, R. AI-Khalifa. Dept. of Pharmacology, College of Medicine, University of Kentucky, Lexington, KY 40536.

Previous studies have demonstrated that the hallucinogen methylenedioxyamphetamine (MDA) enhances the acquisition of conditioned responses (CRs) during Pavlovian conditioning of the rabbit. Further, administration of MDA to rats was associated with enhanced expression of CRs, indicating a release of dopamine (DA) from the ventral tegmental area (VTA). In the present study, rats were exposed to 80 presentations of a 3-way clicker (LO) stimulus and the other a tone (T), for 5 40-min sessions (S). After 24-hr extinction training, rats were exposed to 80 presentations of LO (on a random time 45-s schedule) paired with food. Testing consisted of two 25-s test trials with 10 trials at the present results showed that the saline group increased responding for 20 more than for T from pre-to post-pairing, indicating that 10 resulted in CR. AMPH. (0.1 to 5.0 mg/kg, ip, 5 min before test Ss) produced a dose-dependent enhancement of responding specifically for the CR. SCH 23390, a D1 antagonist (0.5 g/kg, sc, 2 hr before test Ss), shifted the locus of rise in AMPH dose response toward higher AMPH doses. These results implicate the D1 receptor in reward. (Funded by N.S.E.R.C.).


Central dopaminergic transmission has been implicated in memory processes. The delayed non-match-to-sample radial arm maze (DNMTS-RAM) is a useful tool for studying memory in the rat, allowing for an examination of neurobiological treatments on time-dependent memory processes using within-subject designs. The present experiments examined the effects of several direct acting dopaminergic agents on performance of this task. Pretreatment administration of the dopamine agonists, apomorphine (D1; D2; 0.25, 0.5, and 0.1mg/kg), quinpirole (D2; 0.1mg/kg), or SKF83939 (D1 agonist; 0.5mg/kg) did not affect the rate of responding (choices/choice), but did not affect any index of accuracy at a 1 hr retention interval. Similarly, postrunning administration of quinpirole (0.1, 0.2, 1.0 and 2.0mg/kg), SKF83939 (0.3, 0.1 and 0.01mg/kg) or SCH23390 (D1 antagonist; 0.01, 0.01 and 0.1mg/kg) did not affect accuracy, although quinpirole produced a dose-dependent decrease in response rate, assessed 10 hrs post-treatment, and postrunning administration of the benzodiazepine chlordiazepoxide (CDP) was also tested and produced dose-dependent (0.1-5mg/kg) impairments in accuracy at both a 1 and 4 hr retention interval. The effects of CDP are consistent with evidence indicating that GABAergic agents can influence memory processes. In contrast, the present findings indicate that alterations of dopaminergic neurotransmission are not sufficient to alter the memory processes required for accurate performance of this DNMTS-RAM protocol. (Supported by DA08535 to TCN and an Ill. Dept. Public Health grant to JIC).

SYSTEMATIC ELIMINATION OF DISCRIMINATION CRS MAY PROVIDE A MODEL OF DEPRESSION. L. L. Beggs, S. Barbay*, J. J. Jones, J. Green*, D. Krasenbaum*, T. Palfai* and E. Byer*. Behavioral Neurosciences Lab., Syracuse University, Syracuse, NY 13244.

...two experiments examined possible reasons for impaired adaptation to new home-cage odors previously previously exposed to a D1 antagonist on amphetamine (AMPH) produced rats. In the first experiment, male rats were injected s.c. with either water or the NE antagonist D-259-4 on the day of birth and at the time of weaning were placed in a new bedding type in either small or large cages. Ofifactory presentations were made to 10 days old rats, but rats housed in small cages preferred the old bedding odor to the new one, and failed to adapt. All other groups showed no preference. In a second experiment, normal infant and juvenile rats preferred soiled nest shavings from control rats, to shavings which had housed D1 receptor rats. The results suggest that D1-receptor rats produce abnormal odors which, when concentrated in a small area, may interfere with olfactory learning. Supported by National Institute grant number 89088031 to CC-J.
557.11

To study the role of brain adrenergic systems in brain DNA synthesis (BDS), adult male Sprague-Dawley rats were randomly assigned to one of six groups. The control rats received the radioactive precursor only or the drug's vehicle two hours before the precursor. The experimental rats received at time 0 the a-adrenergic antagonist phenolamine (5 μg), the β-adrenergic propranolol (10 μg), the a- and β-adrenergic antagonist yohimbine (1 μg) or the β-adrenergic-isoproterenol (12.5 μg). All rats were given 2 h later 50Ci ³H-thymidine intraventricularly and sacrificed 0.5h later. BDS was measured in several brain regions by the incorporation of ³H-thymidine into DNA. The vehicle decreased BDS in neocortex, and increased it in the septum, neostriatum, hypothalamus, cerebellum and midbrain. In comparison with the group receiving the drug vehicle, a- and β-adrenergic antagonists induced several significant effects, depending on the brain region. The results suggest a modulatory role of central adrenergic systems in brain DNA synthesis. (Supported by CNR and MURST 40% grants).

557.12

To study the role of brain adrenergic systems in brain DNA synthesis (BDS), adult male Sprague-Dawley rats bilaterally lesioned in the dorsal noradrenergic bundle (DNB) by injection of 6-OH dopamin were sham-lesioned. One week later they were given at time 0 the a-agonist phenylhexamine (1 μg), the β-adrenergic-isoproterenol (12.5 μg), or the drug's vehicle. Two hours later all rats received an intraventricular injection of 50Ci ³H-thymidine, and were sacrificed 0.5h later. BDS was measured in several brain regions by the incorporation of ³H-thymidine into DNA. DNB-lesioned rats showed a higher BDS in the hippocampus, neocortex and hypothalamus which was reversed by the a- or β-adrenergic antagonist yohimbine (10 μg). No effect occurred in the septum, neostriatum, cerebellum and remaining brain. The results suggest (i) a tonic inhibitory effect of DNB on BDS in the hippocampus and, indirectly, in the hypothalamus mediated by a- and β-adrenergic systems; and, (ii) a phasic inhibitory effect mediated by both a- and β-systems in the neocortex, and by the β-system in the cerebellum. Thus, a modulatory role of adrenergic systems on brain DNA synthesis is inferred. (Supported by CNR and MURST 40% grants).

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Prior attempts to induce a long-lasting potentiation of evoked responses in the rat neocortex at high frequency stimulation have met with limited success. Here we report that kindling reliably induces a long-lasting alteration of the cortical-evoked potentials (CEP) of the rat. Male hooded rats were chronically implanted with a bipolar stimulating electrode in the corpus callosum and a bipolar recording electrode in the neocortical sensorimotor area. Two weeks later, a series of four baseline input/output (I/O) curves was recorded, followed by anesthesia and recording over an eight day period. Rectangular 100 μsec biphasic pulses were applied to the corpus callosum (CG) and the neocortex (NG). Baseline CEP activity was recorded using the Discovery software (Brainwave, CO) which allows the discrimination of single unit responses from multiunit responses. The CEPs were differentiated from dentate granule cells (DGCs) and hilar "mossy cells" by the latency, waveform and amplitude differences. The separate compartments from corresponding I/O's were recorded for up to five months. Our results indicate that kindling reliably induces a long-lasting alteration of the CEP of the rat. Male hooded rats were chronically implanted with a bipolar stimulating electrode in the corpus callosum and a bipolar recording electrode in the neocortical sensorimotor area. Two weeks later, a series of four baseline input/output (I/O) curves was recorded, followed by anesthesia and recording over an eight day period. Rectangular 100 μsec biphasic pulses were applied to the corpus callosum (CG) and the neocortex (NG). Baseline CEP activity was recorded using the Discovery software (Brainwave, CO) which allows the discrimination of single unit responses from multiunit responses. The CEPs were differentiated from dentate granule cells (DGCs) and hilar "mossy cells" by the latency, waveform and amplitude differences. The separate compartments from corresponding I/O's were recorded for up to five months. Our results indicate that kindling reliably induces a long-lasting alteration of the CEP of the rat.
558.9
ALTERING THE IMPACT OF EARLY REARING ON THE RAT'S SPATIAL MEMORY WITH PRE- AND POSTNATAL CHOLINE SUPPLEMENTATION. L.C.T. Zwaal, F. Mohammedi and J.J. Adams*. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C.

Recent work suggests that pre- and postnatal choline chloride dietary supplementation during development results in long-term facilitation of working and reference memory in rats (Nech et al., 1989). Stimulation history has also been reported to have long-term effects on spatial competences in adult animals.

The purpose of the present experiment is to examine the effects of pre- and postnatal supplemental choline on the impact of early rearing regimes including dark rearing (DR), and complex rearing (CR). Acquisition and retention of spatial and nonspatial water-based discriminations in CR and DR rats were examined.

Subsequently, these rats were given atropine sulfate and re-evaluated.

Results indicate that both pre- and postnatal choline supplementation enhanced performance on working memory in the spatially oriented task, for rats reared in any environment evaluated. A main effect of enrichment was also obtained. Further, atropine administration was demonstrated to impede the efficacy of working memory in all groups tested. These results suggest that postnatal choline supplementation enhances working memory performance in the adult rat.

558.10

The relation between drug tolerance and recovery of function following brain damage was examined. Rats were tested in a standard passive-avoidance two-compartment box. Ordinarily rats will step from a small lighted compartment to a large dark compartment with short latencies. Step-through latencies are increased by either a single injection of scopolamine hydrobromide or bilateral lesions in the septal area. The increased latencies seem to be the result of increased fear of the new compartment and, therefore, appear to be a form of neophobia.

Rats given repeated injections of scopolamine hydrobromide (2 mg/kg), 2 injections per day for 3 days, show tolerance to the neophobia produced by the drug and if the injections are given prior to surgery, show tolerance to the neophobia produced by the lesions. The data are taken as evidence that tolerance to scopolamine and recovery from septal lesions are mediated by the same mechanism, and that mechanism is in place before the brain is lesioned, the deleterious effects that ordinarily result from the lesion are ameliorated.

558.11
PHYSICAL TOLERANCE (BT) TO MORPHINE IN SPINAL RATS. T. Fidolet and C. Weinmis*, Psychol. Dept., LSU.

Behavioral tolerance refers to the fact that many behavioral drug effects are significantly reduced if animals have practiced the response before they receive the drug. We have studied the neural basis of BT by using the spinal, nociceptive tailflick (TF) reflex. TF pretreatment reduces the analgesic effect of morphine and this decrease is retained for at least 1 day after spinalization. To clarify the stimulus-response conditions required for BT, separate groups of intact rats were preexposed to either thermal TF, mechanical tail pinch (TF), or thermal hot plate (HP) nociceptive stimulation, pseudo TF trials or habituation to the experimental context. All rats were spinalized 24 hr later and tested with the TF the next day, at 30, 60, 120 and 180 min after 3.0 mg/kg, sc morphine. There was an overall decrease in the antinociceptive effect of morphine in the experimental groups relative to nonpretreated spinal rats, but this was only significant for the TF, TP and HP conditions. The data suggest that BT to morphine, assessed with the TF, in acute spinal rats, requires presponse to a nociceptive stimulus but not specific pretreatment with the thermal TF test. Supported by NIS Grant 62445

558.12
EFFECT OF ENRICHED ENVIRONMENT AND MORRIS WATER MAZE TRAINING ON BRAIN CHOLINE ACETYLTRANSFERASE ACTIVITY. B.A. Park, R.A. Pappas, S. Murtha and A. Ally*. Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Meaning Long-Evans rats were raised in an enriched (ENR) or impoverished (IMP) standard cage environment evaluated. A main effect of enrichment was also obtained. Further, atropine administration was demonstrated to impede the efficacy of working memory in all groups tested. These results suggest that postnatal choline supplementation enhances working memory performance in the adult rat.

558.13

In an effort to evaluate the occurrence of behavioral LTP we studied the electrophysiological properties of the perforant path-to-dentate gyrus (PP/ DG) pathway in rats before and after they learned a water maze task that is known to be disrupted by lesions of this circuit. After implantation of a stimulating electrode into the PP and a recording electrode into the hilus they learned a water maze task that is known to be disrupted by lesions of this circuit. After implantation of a stimulating electrode into the PP and a recording electrode into the hilus they learned a water maze task that is known to be disrupted by lesions of this circuit. After implantation of a stimulating electrode into the PP and a recording electrode into the hilus they learned a water maze task that is known to be disrupted by lesions of this circuit.

Cephalic responses in the hemipodid pond snail Lymnaea stagnalis is a 2-4 h sequence of behavioral acts of variable durations that leads to intromission of the penis and subsequent transfer of semen. The latter behavior has a fixed duration of 3-4 min at 20°C. Male copulation behavior can be induced by isolation of the animals for over two days, in which case copulation is reciprocal, i.e. the animals alternate between male and female roles. The neurons that project in the penis nerve have been identified using cobal and nickel-lysine backfills. Neurons in three major clusters were found intracellularly in the apical epidermis that responded to this stimulus. This finding confirms the observations. The clusters are located as follows: 1. the I cluster of the right pedal ganglion; 2. the anterior lobes of the right cerebrum; 3. the right pedal ganglion; 4. the right suboesophageal ganglion Th. The neurotransmitter content of these clusters is at least partially known: they contain serotonin, and the peptides APGWamide and FMRFamide, respectively. We are currently investigating the role of these neurons in penis eversion and retraction. A pair of interneurons located in the pedal ganglia has been identified. This pair modulates the electrical activity in two of the aforementioned clusters (ventral lobe I and cluster). In addition, they cause strong excitation of the metacephalic giant cells involved in feeding and weak excitation of the caudal cells, a command system for egg laying. Based on these effects, they were termed small wide acting pedal neurons (SWAP neurons).

In order to analyze the functioning of the neurons above we have to record from its constituents in the freely behaving animal.


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The egg laying behavior of the pond snail is composed of three phases: Resting, Turning and Oviposition. One of the characteristics of egg laying behavior is the almost total absence of buccal rasping in the 1st. Resting phase (< 50 rasps/10 min.), immediately followed by vigorous rasping (150-100 min.) in the 2nd and 3rd. Turning and Oviposition phases. As shown earlier, this rasping is caused by the coordinated action of feeding from oral nematocysts and radular rasps released from the caudal viscera prior to egg laying, and apparently serves to clean the substrate. We therefore investigated the electrical activity of the metacephalic giant cell (MCG), an interneuron that controls the food finding and egg laying. Animals were equipped with fine wire electrodes on one or both the MCGs, and both behavior and electrical activity were recorded. After the experiment, the recorded spikes were identified as coming from the MCG by recording synchronous spikes in the contralateral MCG. The dramatic change in the rate of rasping during the transition from Resting to Turning phase behavior seen in normal animals was, surprisingly, not reflected in the rate of spiking of the MCG. After oviposition had ended, however, a significant drop in MCG spiking occurred. This low level of spiking was then maintained for many hours. Since these experiments were done in the absence of food stimuli, we became interested in the interaction between food stimuli and egg laying. In the presence of a chemical stimulus (xenobiotics, 3 g/l) control animals raps at high rates (125-150 min) that are maintained for many hours. In egg laying animals, however, the rasping drops to approx. 40 rasps/min immediately after the injection of the first, Resting phase of egg laying. It appears that buccal rasping during egg laying is first suppressed, and then increased in the 2nd and 3rd phases. We are currently investigating the role of the MCG in this suppression of rasping in the presence of food stimuli.

EARTH-STRENGTH MAGNETIC FIELD STIMULI THAT ELICIT SPIKING IN AN IDENTIFIABLE MOLLUSKAN NEURON. Kenneth J. Lohmann and A. O. Dannis Willows, Dept. of Biology, University of Puget Sound, Gig Harbor, WA 98335. The marine mollusk Tritonia diomedea can orient to the geomagnetic field of the earth (Lohmann and Willows, Science, 235: 331-334 (1987)). Two large, identifiable neurons (L Pe 5 and R Pe 5) in the brain of Tritonia respond with enhanced electrical activity to changes in earth-strength magnetic fields (Lohmann et al., in press, J. Exp. Biol). These cells may be components of a neural circuit underlying detection of the geomagnetic field or orientation to it. To study the magnetic field parameters that best elicit responses from L Pe 5 and R Pe 5, we have constructed a computer-controlled coil system consisting of three Rubens cubes coils arranged orthogonally; two coil systems control the horizontal component of the magnetic field while a third controls the vertical component. An earth-strength field can therefore be generated in any direction or rotated to a new position at variable rates during experiments. Responses of L Pe 5 to changes in horizontal fields, vertical fields, field intensity, and inclination angle are compared.

PROTEIN SYNTHESIS BY INDIVIDUAL, PUTATIVELY HOMOLOGOUS NEURONS IN DIFFERENT SPECIES OF NUDIBRANCH MOLLUSCS. C. M. Lohmann* and A. O. Willows. Friday Harbor Laboratory, 620 University Road, Friday Harbor, WA 98250.

Morphological and behavioral considerations suggest that specific neurons in different species of sea slugs may be evolutionarily homologous. We have attempted to test this possible homology by using gel electrophoresis to study protein synthetic patterns of several identifiable neurons from four nudibranch species. Four experiments that we have examined possess a large, white (under epification) neuron in the pedal ganglia similar to Pedal 5 of Tritonia diomedea. Analysis of Pedal 5 neuron and 5's putative homologous shows that all produce one major and several minor bands near 45-55 kD. The results thus indicate that neurons of similar morphology have similar protein banding patterns and support the hypothesis of evolutionary homology. Thetical findings from biochemical and neurophysiological data, imply that 1) similar behavior in different species may be mediated by homologous neurons and 2) differences in behavior may involve different uses of the same neurons.
559.7

Decapod cephalopods, such as cuttlefish and squids, are able to move (pitch, roll, and yaw) their head relative to the body. However, no data exist on possible receptors that detect such movements. Using morphological techniques (L.M., TEM, SEM), we describe, for the first time, a receptor organ that possibly serves as a neck proprioceptor organ.

In the squid Lolliguncula brevis, epidermal hair cells exist on the dorsal side of the neck under the mucal cartilage, close to the animal’s midline on either side (left and right) of the nuchal crest. The cells can be divided into 4 groups, each with 20-30 presumably primary sensory cells. Each cell carries 30-300 kinocilia, arranged in an elongated ciliary group, with a morphological polarization parallel to either the longitudinal or the transverse axis of the animal. The hair cells are innervated by a branch of the posterior nerve. Cobalt tracking shows that all hair cells project ipsilaterally (and perhaps contralaterally) to one brain area only, the ventral part of the ventral magnocellular lobe. This area receives afferents from the equilibrium receptor systems as well.

In summary, the data indicate that the epidermal neck hair cells are sensory in nature and presumably serve to monitor the position of the head relative to the body.

Support by NHI (HAR 5 801 EY 08312-02), and German LFGP and DAAD Grants.

559.8
TEMPORAL SELECTIVITY AND RESOLUTION IN THE AUDITORY SYSTEM OF THE GREEN TREEFROG (HYLA CINEREA). S.E. Allan* and A.M. Simmons. Psychology Department, Brown University, Providence, RI 02912.

Differences in rate of amplitude modulation (AM) carry biologically relevant information in many animals. These may be reflected in the temporally resolving power of their central auditory system. Multi- and single-unit activity were recorded in the torus semicircularis (TS) of hyla cinerea in response to AM noise stimuli varying in rate and degree of modulation. Selectivity to AM rates and the ability to resolve depth of AM were examined by changes in firing rate and synchronization to the modulation frequency. Different types of filter functions for AM were found. These include temporally-tuned units with AMs from 0 Hz and 300 Hz, which correspond to the natural AM rates of the species' vocalizations. A characteristic dip in sensitivity at 100 Hz, the AM rate of a sympathetic species' call, was also apparent. Comparable threshold functions were found using a psychophysical technique. Temporal modulation transfer functions were low-pass in shape, with thresholds reaching 100% depth at AM rates above 100 Hz. The minimum integration time calculated from these functions ranged from 1.0 - 2.5 ms and the difference limens for detectable amplitude changes at the best AM rates varied between -1.36 dB - 1.09 dB. (Supported by NIH, NSF, and DHP)

559.9

Male Xenopus laevis frogs attract females with rapidly alternating fast and slow trills known as mate calls. The clicks that make up the fast trill portion become progressively louder whereas the clicks of female-typical release calls- ticking - are not amplitude modulated. Sex differences in synaptic efficacy at the laryngeal neuromuscular junction contribute to amplitude modulation by regulating the number of contracting muscle fibers. We thus wished to determine whether amplitude modulation is critical to the attractiveness of male song. An artificial mate call in which the fast trill portion was of uniform amplitude was used to measure the female's phonotaxic response. Receptive females turn towards a posterior test of significance. Thirty-eight POA units and 10 (26.3%) of which increased their firing rate in response to the MC stimulus. Nine (39.1%) of 23 PST histograms were generated to examine spike rates and modulation. Selectivity to AM rates and the ability to resolve depth of AM were examined by changes in firing rate and synchronization to the modulation frequency. Different types of filter functions for AM were found. These include temporally-tuned units with AMs from 0 Hz and 300 Hz, which correspond to the natural AM rates of the species' vocalizations. A characteristic dip in sensitivity at 100 Hz, the AM rate of a sympathetic species' call, was also apparent. Comparable threshold functions were found using a psychophysical technique. Temporal modulation transfer functions were low-pass in shape, with thresholds reaching 100% depth at AM rates above 100 Hz. The minimum integration time calculated from these functions ranged from 1.0 - 2.5 ms and the difference limens for detectable amplitude changes at the best AM rates varied between -1.36 dB - 1.09 dB. (Supported by NIH, NSF, and DHP)

559.10
ACOUSTIC NICHE PARTITIONING BY THREE SPECIES OF NEOTROPICAL PROCS. B.S. McClelland, W. Wilczynski, J.M. Wicks, and A.S. Rand. Dept. of Psychol., Univ. of Texas, Austin, TX, 78712, and Smithsonian Tropical Res. Inst., Balboa, Panama.

Three closely related frog species, Hyla ebraccata, H. philobates, and H. microcephala, breed simultaneously and can be found in mixed choruses. We investigated acoustic communication in these species in a mixed chorus in Gamboa, Panama. All have high frequency, predominately advertisement calls, but mean dominant frequency differs among the species: H. e.: 3.2 kHz; H. p.: 3.8 kHz; H. m.: 6.1 kHz. H. e. and H. p. calls differ in temporal structure: H. e.: 2.8 kHz; H. p.: 5.3 kHz. Thus these species partition the acoustic channel used for communication in the frequency domain and, in the two species (H. e. and H. p.), 8N and TS sensitivity measures in the AP range. In the BP range, both SVL and CP sensitivity, and HCBP correlates with no sensitivity measure. Both are largest in H. e., intermediate in H. p., and smallest in H. m., as would be expected if these features contribute to the resonance of the ear. Additional studies are underway to determine if call differences are reflected in species differences in laryngeal structure.

Supported by the Smithsonian Institution and NIMH 801 MH 45350. We thank D.L. Greene for assistance.

559.11
ACOUSTIC MODULATION OF HYPOTHALAMIC FUNCTION IN HYLA CINEREA. J. D. Allison. Dept. of Psychology, Univ. of Texas, Austin, TX, 78712.

Previous studies reveal that the preoptic area (POA) and ventral hypothalamus (VH) of anurans receive input from thalamic and midbrain auditory nuclei. We examined the responses of POA and VH units to acoustic stimuli using standard electrophysiological techniques. Spontaneously active units were isolated and then stimulated with white noise at high levels. The unit's response to the stimulus was measured using a Tukey HSD ANOVA and post-hoc test of significance. Thirty-eight POA units were examined, 10 (26.3%) of which increased their firing rate in response to the stimulus. Nine (39.1%) of 23 POA units examined increased their firing rate in response to the MC stimulus. Nine (39.1%) of 23 PST histograms were generated to examine spike rates and modulation. Selectivity to AM rates and the ability to resolve depth of AM were examined by changes in firing rate and synchronization to the modulation frequency. Different types of filter functions for AM were found. These include temporally-tuned units with AMs from 0 Hz and 300 Hz, which correspond to the natural AM rates of the species' vocalizations. A characteristic dip in sensitivity at 100 Hz, the AM rate of a sympathetic species' call, was also apparent. Comparable threshold functions were found using a psychophysical technique. Temporal modulation transfer functions were low-pass in shape, with thresholds reaching 100% depth at AM rates above 100 Hz. The minimum integration time calculated from these functions ranged from 1.0 - 2.5 ms and the difference limens for detectable amplitude changes at the best AM rates varied between -1.36 dB - 1.09 dB. (Supported by NIH, NSF, and DHP)

559.12
MORPHOLOGICAL CORRELATES OF AUDITORY SENSITIVITY IN AMPHIBIANS. J. H. Fox. Department, Brown University, Providence, Rl 02912.

Comparative studies involving anuran audition are often limited by the paucity of threshold data that have been reported. Certain well documented morphological characteristics may correlate with auditory sensitivity and may therefore be useful as sensitivity indices, enabling comparative work with more diverse taxa. In this study, a database was compiled, and the relationships between auditory sensitivity measures in the AP range. In the BP range, both SVL and CP sensitivity, and HCBP correlates with no sensitivity measure. Both are largest in H. e., intermediate in H. p., and smallest in H. m., as would be expected if these features contribute to the resonance of the ear. Additional studies are underway to determine if call differences are reflected in species differences in laryngeal structure.

Supported by the Smithsonian Institution and NIMH 801 MH 45350. We thank D.L. Greene for assistance.
559.13
SPECTRAL AND TEMPORAL CUES PRODUCED BY THE EXTERNAL EAR OF THE BIG BROWN BAT, Eptesicus fuscus, TO CLOSED FIELD DICHOTIC STIMULATION. T. Haresign, M. Perragut, and J. Simons. Hunter Laboratory, Brown University, Providence, R.I. 02912

Extracellular recordings were made from the inferior colliculus (IC) and auditory cortex of Eptesicus fuscus. Using a closed field dichotic stimulation system, units were tested to determine their best frequency (BF), latency, interaural intensity difference (IID) tuning, and interaural time difference (ITD) tuning. Units were functionally classified according to their binaural response properties. The most common type of IID tuning among the units tested is ET tuning (contralateral ear excitatory, ipsilateral ear inhibitory). In the IC there is a wide distribution of BF’s and latencies, suggesting that one of the functions of the IC may be to act as a latency disperser. Many of the ET units are sharply tuned for frequency and show a sharp boundary between excitation and inhibition in their IID tuning curves. These units should be very sensitive to spectral differences between the ears.

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559.15
A COMPUTER SIMULATION OF THE UNIFIED PERCEPTION OF TIME AND FREQUENCY BY THE BIG BROWN BAT. P.A. Salamant, J.A. Simons, and S.P. Dean. Dept. of Psychology and Section of Neurobiology, Brown University, Providence, RI 02912

A newly developed Spectral Correlation And Transformation algorithm (SCAT) has demonstrated the ability to reproduce the performance of the big brown bat, Eptesicus fuscus, in behavioral experiments designed to measure glint resolution, phase sensitivity and amplitude latency tradeoff. The SCAT algorithm is designed around known physiological properties of inner hair cells, delay-tuned neurons and other components of the bat auditory system, and in addition, incorporates novel, physiologically motivated, processing elements. Like Eptesicus, the SCAT algorithm can detect the separation between each pair of glints in targets separated by as little as 4 μs. Jitter experiments in which the phase of the returning echo is inverted by 180° show that bats perceive the inverted correlation function of the emission and echo. This effect is reproduced by SCAT as a result of the temporal properties of delay-tuned NM neurons responding to dominate peaks of the group delays in each filter bank. Amplitude-latency trading experiments, in which the temporal and spectral contributions to complex image formation can be dissociated, have also been replicated by SCAT showing that the novel transforms it employs can help facilitate our understanding of auditory perception in bats. (Supported by NIMH Grant MHL8882, ONR grant N00014-89-J-3055 and NIMH grant MHR9115)

559.16

We photographed foraging Noctilio leporinus in Costa Rica and recorded their echolocation signals simultaneously. For the "flapping search flight", the bats fly about 50 cm over water emitting short pulses of 2-3 CF and CF-MF pulses of up to 14 ms long. In the extended leg search flight, the bats fly only a few cm over water with their legs hanging. Eventually they lower their feet to dip into or to rake through the water emitting groups of short CF-MF signals interspersed with 1-3 longer CF and/or CF-MF signals. The bats use three hunting strategies. The pointed dip is directed to a single target to scoop it out of the water. At pointed dips out of the extended leg search flight, the bats do not react to the target in their echolocation behavior. However, at pointed dips out of the flapping search flight, which we evoked with a water jet simulating a jumping fish, the bats reacted with a reduction in pulse duration and interval, finally producing short FM signals. At the directed rake, the bats rake through patches of many jumping fish with no reaction in their echolocation behavior. Therefore we think that they do not localize a single fish, but get accurate information to direct their flight to the area with high jumping activity. The random rake strategy is used if there are no jumping fish around. The bats make very long rakes in areas which previously had many jumping fish. As the bats have no way of knowing if fish are still there, we think that they catch fish only by chance. The bats need 50-200 dips or rakes to catch a fish. (Supported by DFG/SSR 307)

559.17
LEARNED RELEVANCE OF SPECIES-SPECIFIC VOCALIZATIONS AND THEIR 14C-2-DG PATTERNS IN AUDITORY CORTEX IN THE DEGU (OCTODON DEGUS) S. Braun*, D. Bonke1, and H. Scheich. Institute of Zoology, TU-Darmstadt and CE Merck1, D-6100 Darmstadt, Germany

In a Chilenian rodent, the degu, early learning of species-specific vocalizations during nursing and offspring care was investigated. Degus are highly social and live in large family structured colonies. Dego mothers vocalize while nursing their pups, which are precocial at birth. Behavioral studies of degu pups revealed an approach to mother calls while pups raised by muted mothers failed to react. This leads to the assumption that the behavioral significance of the calls is learned early during nursing. Temporal and spectral cues changed systematically with the position of the sound source. Measurements made after the removal of the tragus show that monaural cues for elevation appear to be produced by pinna-tragus reverberations. The information available to the bat and represented in the transfer functions and impulse responses has been shown to have behavioral relevance for the bat in sound localization experiments.

Supported by NIH Grant #NIDCD DCO0511

559.18
SELECTIVE RESPONSES OF MACAQUE TEMPORAL NEURONS TO COMPLEX SOCIAL STIMULI. L.B. Brothers & D.D. Ring. UCLA-Sepulveda VA Medical Center & UCLA Brain Research Institute, Sepulveda CA 91343.

The present study extends previous observations in medial amygdala and adjacent cortex of neurons selective for social features. Moving visual and/or auditory stimuli lasting 25's were presented to an alert subject while recording in the region of the right amygdala. Stimuli were drawn from an extensive library of macaque behavior stored on laser disk. Neuronal responses were compared to responses elicited by relevant control stimuli differing from stimuli along one or more dimensions. Of 533 cells analyzed, 14 showed selective responses to features of presented stimuli, for example, agonistic content. The response of one cell to segments depicting a retreating animal was significantly enhanced by concomitant presentation of the animal's vocalizations. Such neurons may participate in integrations necessary for forming high-level social representations.

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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991

559.19


Ethology and behavior ecology studies are often interested in behavioral adaptations; however, they often model behavior as being ideally or optimally conditioned. In other words, they rarely consider the existence of neuromuscular physiological components that restrict the evolutionary potential of behaviors. We believe that behaviors are suboptimally adapted as a result of various physiological constraints. The current study was undertaken to provide insight in this regard.

Six giraffes, 4 okapis, 4 roe deer, 6 kangaroos, 4 red pandas, and 3 raccoons) were videotaped for 10 hr periods on 4 separate occasions over a 1.5 yr period. Every behavior pattern occurring during each 10 hr videotaping session was classified in such a way that cross-species comparisons could be made. Behavior pattern durations and occurrences throughout the 10 hr periods were then analyzed to determine what features were similar among all 6 species.

The results indicate that: 1) over 80% of the behavior patterns were similar in all 6 species, 2) of these patterns, their relative frequency of occurrence was similar in all 6 species, 3) a temporal hierarchy of behavior patterns was similar in all 6 species, with spectral peaks occurring near 0.5, 3-6, 1-2 min, 10-20 min, 1-1.5 hr. The results of this study support the hypothesis that many aspects of behavior organization are conserved in the mammalian lineage, indicating potential physiological restrictions on the evolutionary potential of behavior. We acknowledge the assistance of the San Diego Zoo and Wild Animal Park, Fish Aquatic Sanctuary, Phoenix Zoo staffs. This study was supported by PHS Grant DE00205.

559.20


A computational model of nervous system function during classical and instrumental conditioning is proposed. The model assumes the form of a network of control systems. Each control system is capable of learning and is referred to as an associative control system (ACS). Learning systems consisting of ACS networks, employing the drive-reinforcement learning mechanism (Klopf, 1988) and interacting in a real-time, closed-loop fashion with environments, are capable of being instrumentally conditioned, as demonstrated by means of computer simulations. The simulated systems learn, in multiple-step mazes, to chain responses that eventually lead to primary positive reinforcement. The systems also learn to avoid primary negative reinforcement. The temporal order in which the responses are learned is consistent with that observed in animal learning. Also, consistent with animal learning experimental evidence, the ACS network model accounts for a wide range of classical conditioning phenomena. The ACS network is intended as a model of limbic system, hypothalamic, sensory-motor function, suggesting a relationship between classical and instrumental conditioning that is consistent with Nower's (1969) two-factor theory of learning.
560.5 DESCENDING SENSORY INFORMATION PROJECTS HOMOTOPICALLY TO THE ELECTRORECEPTIVE DORSAL OCATOLOID NUCLEI IN SKATES

R.A. Conley and D. Rednick
Wesleyan University, Biology, Middletown, CT 06457

The dorsal granular ridge (DGR) in elasmobranchs is a part of the vestibulolateral cerebellum which projects via parallel fibers to the dorsal octavolateralis nucleus (DON), the primary electrosensory nucleus. Somatotopic representations of electrosensory and proprioceptive information in DGR and the topography of the DGR to DON projection suggests that the projection is homotopic. A homotopy has now been demonstrated by activating the parallel fibers with a stimulating electrode placed in DGR. Evoked potential and unit responses were recorded electrophysiologically in DON.

The evoked potential is characterized by a positivity at 4 ms latency followed by a negativity. The positivities are believed to be synchronically induced depolarizations on the dendrites of the DON ascending efferent neurons. Upon parallel fiber activation, typically one or two spikes followed by a long-lasting suppression are elicited in the ascending efferent neurons. Responses to electric fields are reduced in magnitude when presented during this suppression period.

Peak evoked potential amplitudes and unit responses to parallel fiber activation occur only in areas of the DON containing units whose receptive fields correspond with the composite receptive field recorded at the stimulation site in DGR. The data show a homotopic projection of information from DGR onto DON.

560.6 LAMINAR SEGREGATION OF FUNCTION IN THE TORUS SEMICIRCULARIS OF WEAKLY ELECTRIC FISH.
S J. Call and G J. Rose. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112

The processing of sensory information in laminated structures is a common feature of vertebrate central nervous systems. To understand the laminar organization, we studied the processing of electrosensory information in the dorsal torus semicircularis of Eigenmannia. This region of the midbrain consists of 12 laminae and approximately 55 neuron types.

We investigated structure-function relations in the torus by labeling physiologically characterized neurons with Lucifer Yellow. Ampullary and tuberous information is processed differentially within the lamina of the torus: laminae 2 and lamina 5 are predominantly ampullary and tuberous in function, respectively.

Ampullary units respond well to "beats," i.e., modulations of signal amplitude and phase, while from the interaction of foreign signals with the fish's own quasi-sinusoidal electric organ discharge. The "beat rate" is proportional to the frequency difference between these two signals. Units respond well to low best rates but poorly to high rates (i.e., "low-pass" temporal filtering).

We observed the temporal filtering in the membrane potentials of neurons that reflect the patterns of modulations of signal amplitude and phase. Changes in the structure of these pops that accompany changes in the beat rate have provided insights into the mechanism of temporal filtering. Models of these mechanisms will be presented. Supported by grants from NSF and Sloan Foundation.

560.7 LONGITUDINAL TRACKING RESPONSES OF GYMNOTIFORM FISH, G. J. Rose and J. C. Canfield. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84122

A variety of behaviors serve to stabilize sensory images on receptor arrays. In one class of these behaviors, global movements of the "surround" elicit compensatory movements of the receptive surface. Previous studies have shown that weakly electric fish are able to track aggregate lateral movements of surrounding objects exclusively by the electric sense.

We now report measurements of longitudinal tracking responses of Eigenmannia and Sternopygus. These movements were elicited by moving a "shuttle" in which the fish was situated. Fish were able to track longitudinal motion of the shuttle using visual or electrosensory cues.

The gain and phase of tracking responses were studied as a function of the magnitude and frequency of the shuttle motion. Fish were able to track velocities of shuttle motion of at least 4 cm/s with little decrement in gain.

We delivered signals that excited ampullary receptors, tuberous receptors, or both during longitudinal tracking of various velocities of shuttle motion. The jamming effects of these stimuli will be presented. Supported by grants from NSF and Sloan Foundation.

560.8 A SUBLEMNISCAL PREPACEMAKER NUCLEUS IN GYMNOTIFORM ELECTRIC FISH DEPOLARIZES RELAY CELLS OF THE PACEMAKER NUCLEUS VIA NMDA-TYPE RECEPTORS. C H. Keller, M Kawasaki, W. Heiligenberg. Scripps Inst. Oceanogr., UCSD, La Jolla, CA 92039; and Inst. of Neurosci., U. of Oregon, Eugene, OR; Dept. Biology, U. of Virginia, Charlottesville, VA.

The medial pacemaker nucleus of gymnotiform fish is an endogenous oscillator which triggers each electric organ discharge (EOO) by a single command pulse. The pacemaker nucleus consists of electrosensory cells which generate the rhythm, and 'relay' cells, which follow the pacemaker cells and excite the motor neurons of the electric organ. By injecting chloraloxin into the pacemaker nucleus, we have recently labeled a hitherto unknown sublemniscal nucleus which, in addition to the classical diencephalic pacemaker nucleus, provides input to the pacemaker nucleus.

Stimulation of the sublemniscal nucleus in the genera Sternopygus and Hypopomus causes a sustained depolarization of the relay cells that shut down the regular electric organ discharges over a period of several seconds. Pressure injection of the NMDA receptor blocker, APV, to the pacemaker nucleus reversibly prevents this response while not affecting other forms of modulation of the pacemaker that are not mediated by NMDA.

The same sublemniscal nucleus was also labeled in the genera Eigenmannia and Paragramphorus, but its functional significance has yet to be determined.

560.9 ULTRASTRUCTURAL EVIDENCE OF GABA-ERGIC INHIBITION AND GLUTAMINE-ERGIC EXCITATION IN THE PACEMAKER NUCLEUS OF THE GYMNOTIFORM ELECTRIC FISH, HYPOPOMUS, G. Kennedy and W. Heiligenberg. Neurobiology Unit, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92039

The medullary pacemaker nucleus of Hypopomus is an endogenous oscillator which triggers each electric organ discharge (EOO) by a single command pulse. The pacemaker nucleus consists of electrosensory cells which generate the rhythm, and 'relay' cells, which follow the pacemaker cells and excite the spinal motor neurons of the electric organ. The pacemaker cells receive two inputs: one synaptic and independent of the complex of the diencephalic pacemaker nucleus, a GABA-ergic inhibition and a glutamine-ergic excitation. Whereas the downstroke of the diermatically, the pacemaker cycle, the excitation accelerates the pacemaker cycle in a smooth and gradual manner.

We have labelled the two inputs to the pacemaker nucleus anterogradely by injecting HRP to the respective sites of the pacemaker complex. By using respective immunoprophased antibodies and en-grind staining techniques, we then demonstrated GABA and glutamate in labelled synaptic profiles of ultra-thin sections of the pacemaker nucleus. The two types of synapses were interspersed on the surfaces of somata and dendrites of pacemaker cells.

560.10 MIDBRAIN AND DIENCEPHALIC LINKS WITHIN THE NEURAL NETWORK UNDERLYING THE JAMMING AVOIDANCE RESPONSE IN EIGENMANNIA. Walter Metzner and Walter Heiligenberg. UCSD A-002, UCSD, La Jolla, CA 92039

We now report measurements of longitudinal tracking responses of Gymnotiform fish. In one class of these behaviors, global movements of the "surround" elicit compensatory movements of the receptive surface. Previous studies have shown that weakly electric fish are able to track aggregate lateral movements of surrounding objects exclusively by the electric sense.

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AFRICAN ELECTRIC FISH, GYMNASCHUS, USE THE IDENTICAL COMPUTATIONAL ALGORITHM AS SOUTH AMERICAN ELECTRIC FISH FOR THEIR JAMMING AVOIDANCE RESPONSES. Martina Hadley*, L. Malin, R. Ouirion2 and S. Kar2. 1 Dept. of Anatomy, University of Oslo, 2Dept. of Anatomy, University of Ottawa, Ontario, Canada. K1H 8M5, and 2Douglas Hospital Research Centre, Verdun, Quebec, Canada H4H 1R3.

A conspicuous electric organ differs from the median electric organ of elasmobranchs. The EOD is controlled by the medullary pacemaker nucleus (PM) whose only known input is from the dipeptic pacemaker nucleus (PPN). The PPN’s output is tonically powerful, but in the frequency of the EOD, called “chirps”, which are used in communication. Immunohistochemical studies revealed a major system of substance P-like immunoreactivity (SPLI) which originated in the hypophyseal axis and, in males only, innervated many areas of the diencencephalon, including the PPN, its ventral territory (VT, an area containing dendrites of PPN cell bodies) and the hypothalamic nuclei. Hypothalamic SPLI innervation of the diencencephalon was absent in females. Our results suggest that a SP system of hypothalamic origin is involved in regulating both endocrine events and sexual/territorial-related behavior in males. No sexual dimorphism of SPLI was found in the amygdala, mesencephalon or rhombencephalon. Neuropeptides SP-li and NK1 receptors were found in the brain by autoradiography; their distribution showed a very close correlation with that of SPLI, in particular the NK1 receptor, which preferentially binds SP, was found in the VT.

Neuroactive substances were injected into the PPM of curarized, respirated fish to evoke chirps. Glutamate evoked short chirps (5-30 sec) similar to spontaneous chirps. Substance V-evoked chirps were longer (25-60 sec) and had a more complex waveform. Glutamate was most effective in the PPN proper. In contrast, SP was most effective in the VT of the PPN, an area with abundant dendrites from PPN cell bodies, dense SPLI and NK1 receptors.

This research was supported by grants from the Medical Research Council of Canada to LM and RQ.

THEIR JAMMING AVOIDANCE RESPONSES. Masashi Kawasaki. Department of Neurobiology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan. J. Neurophysiol. 56 (1986) 1323-1334. The JAR (Jamming Avoidance Response) was first described in the South American electric fish. Raja erinacea, where the JAR is produced by a pair of electric organ discharges (EODs) at a constant frequency which is used in electrolocation. The EOD is controlled by the medullary pacemaker nucleus (PM) whose only known input is from the dipeptic pacemaker nucleus (PPN). The EODs consist of chirps of different frequency, which may expose them to predation.

Neuroactive substances were injected into the PPM of curarized, respirated fish to evoke chirps. Glutamate evoked short chirps (5-30 sec) similar to spontaneous chirps. Substance V-evoked chirps were longer (25-60 sec) and had a more complex waveform. Glutamate was most effective in the PPN proper. In contrast, SP was most effective in the VT of the PPN, an area with abundant dendrites from PPN cell bodies, dense SPLI and NK1 receptors.

This research was supported by grants from the Medical Research Council of Canada to LM and RQ.

EVIDENCE FOR DISTINCT BUT OVERLAPPING POPULATIONS OF COMMISSURAL AND GABA-IMMUNOREACTIVE NEURONS IN THE MEDULLARY ELECTROSENSORY NUCLEUS OF THE LITTLE SKATE, RAJA ERINACEA. C. J. Dungan and R. J. Danks. Dept. of Biology, Wesleyan University, Middletown, CT 06459.

Activity of neurons of the dorsal octavo-lateralis nucleus (DON), the primary electroreceptor nucleus in elasmobranchs, is modulated by electrical interference from the animals own ventilation, but to a much lesser degree than that of the primary afferent fibers. There is physiological evidence that inhibitory commissural connections between the bilateral dorsal nuclei are part of a mechanism for rejecting common-mode noise signals such as ventilatory interference (New and Bodznick, 1990). We have begun anatomical characterization of the commissural pathway. Retrograde transport of horseradish peroxidase or fluorogold injected into the DON resulted in labelling of commissural cells in both central and peripheral zones of the commissural DON. Double-labelling studies indicated that a population of commissural cells in the central zone labelled by retrograde transport of fluorogold, also show GABA-immunoreactivity. GABA-immunoreactivity was also displayed in non-commissural central zone cells and in stellate cells of the molecular layer of the DON. The doubly labelled cells are likely candidates for mediators of a common-mode noise rejection mechanism in the DON.

NEUROTHEOLOGY AND VISION IN THE BIOLUMINESCENT FISH, PORICHTHYS NOTATUS. A. F. Mensing and J. P. Case. Sam Houston State Univ., Nashville TN 37240 and Univ. of California, Santa Barbara, CA 93106.

The midshipman fish, Porichthus notatus, is a nocturnal predator that feeds on luminous organisms. Fish were presented with photic stimuli characterized by luminance, intensity, wavelength, duration, and location. Fish were observed in the dark, illuminated by specific flashes of specific intensity (450-750 nm), wavelength (0.02-0.07 Hz) and duration (250-750 ms). Higher intensity flashes (>2500 ms) evoked negative responses while intermediate values yielded graded reactions. Increasing source intensity while maintaining equal intensity, reduced number of positive responses. Negative stimuli elicited faster responses.

Juvenile P. notatus must acquire exogenous sources of luciferin to remain luminous. Fish are attracted to photic signals characteristic of their predators and avoid light that may expose themselves to predation.


Smolt transformation (ST) involves major changes in the physiology and behavior of salmon. During ST the fish change from territorial to schooling behavior, start seaward migration, and form an olfactory system. To identify the central control systems that regulate ST, we have traced the neuronal connections of the retina, the optic tectum, the photoreceptor plexus, the olfactory projections and the hypothalamic centers by means of DiI tracing. The results were then correlated with immunocytochemically defined neuropeptides and peptide-like immunoreactivity.

The most striking results were (1) the extensive retinohypothalamic projection, (2) the absence of a cyto- or chemoarchitectonically circumscribed nucleus containing the supraoptohypophyseal nucleus in mammals, (3) the extensive pineal projections to the dorsal thalamus and pons, (4) a close association of a dopamine neuron population with afferent axons from both the retina and pineal organ, (5) DA neurons in the hypothalamus and hypothalamic and periventricular nuclei that project to the optic tectum, (6) a close association of DA neurons in the telencephalon with the projections to the olfactory projections. In all, our results indicate a central role for dopamine in the central processing of sensory information known to be essential for several aspects of smolt transformation in salmon.

Supported by the Swedish Natural Science Research Council (NFR), the Swedish Forestry and Agricultural Research Council (SJFR) and the National Institutes of Health and the Alaska Sea Grant Program.
TASTE MEDIATES INNATE FEEDING BEHAVIOR WHILE OLFACTION FACILITATES CONDITIONED DISCRIMINATION OF AMINO ACIDS BY CATFISH. T. Valentine (University of Ljubljana, Yugoslavia), S. Wegele and J. Caprio (Louisiana State University)

The inhibition of feeding in channel catfish (Ictalurus punctatus) by the long-term central escape state was reduced by maintaining animals individually in aquaria with black gravel, by regular feeding and by the long-term avoidance of visual stimuli which release escape behavior. Naïve channel catfish responded to L-alanine and L-arginine (>1μM) and L-proline (>1μM) with a substantial degree of population spike (100.96 ± 35.16%), in comparison to baseline. In contrast, L-proline, which is not a substrate for the fish's main amino acid transporter, did not produce LTP (0.92 ± 1.56%) in acute control animals or subsequent conditioning trials.


Supported by the NHMRC of Australia

561.2


Glucocorticoids mediate a host of effects in catfish and other teleosts through the GABA-A/benzodiazepine receptor. While it has been shown that glucocorticoids can alter neuronal activity through intracellular receptor-mediated changes in protein synthesis and through poorly understood interactions with receptors on neuronal membranes, recently we characterized a high-affinity corticosteroid receptor in synaptic membranes from an amphibian brain, Taricha granulosa (Orcinrich et al. in press, Science). The receptors were localized autoradiographically in the neuropil in discrete brain regions and they appear to mediate rapid behavioral responses to corticosterone (CORT). Since the binding site is not linked to G-PROTEIN.

We here present dose response studies on the effect of food deprivation, ADX and adal teronal activity through intracellular receptor-mediated changes in protein synthesis and through poorly understood interactions with receptors on neuronal membranes. Recently we characterized a high-affinity corticosteroid receptor in synaptic membranes from an amphibian brain, Taricha granulosa (Orcinrich et al. in press, Science). The receptors were localized autoradiographically in the neuropil in discrete brain regions and they appear to mediate rapid behavioral responses to corticosterone (CORT). Since the binding site is not linked to G-PROTEIN.
**561.5**

THE RELATIONSHIP BETWEEN HIPPOCAMPAL LESIONS AND GLUCOCORTICOID LEVELS IN AN ANIMAL MODEL OF WANDERING IN ALZHEIMER'S DISEASE.


Dementia was induced in 33 Long-Evans rats by the administration of bilateral cerebral ventricular injections of Aminooxyacetic Acid (A0A) at 3-month intervals. Following a 2-month recovery period, the animals were divided into three groups: intact controls (IC), lesioned (L), and lesioned and chronically treated with dexamethasone (L+D). The L group received lesions and were treated with dexamethasone every other day for 12 weeks, whereas the L+D group received lesions and were treated daily with dexamethasone for 12 weeks.

**561.6**

STRESS INDUCED CORTISOL SECRETION INFLUENCES INFORMATION PROCESSING IN MN, G. Fern-Wolfsohn, D. Nagel* and H. Feldt*, Dept. of Med. Psychology and Internal Medicine, University of Ulm, D 7900 Ulm, Germany.

Glucocorticoids are assumed to exert differential effects on central nervous function through different receptor types described in rat brain (de Kloet & Reul, 1987). In a new experiment we studied the influences of hydrocortisone, which increases cortisol levels, as opposed to dexamethasone, which suppresses endogenous cortisol secretion, on sensory functions. In auditory tasks opposite effects of these steroids were most prominent on stapedial reflex: higher cortisol levels reflex needed more dB to be elicited. In the present experiment we studied this effect during the Morris water maze. In 24 subjects repeated measurements of stapedial reflex and salivary cortisol levels were carried out in parallel. Unexpectedly ss were asked to deliver a videotaped speech in bright light which led to a significant decrease in cortisol levels in most of the ss. A second control session followed the identical time course without speech. Significantly more dB were needed to elicit stapedial reflex at the time of cortisol maximum as compared to the minimum after recovery from stress 100 minutes later or to control session. Our results suggest that high glucocorticoid secretion attenuates perceptual abilities.

**561.7**

AROMATASE ACTIVITY IN PREOPTIC AREA, HYPOTHALAMUS, AND AMYGDALE OF INTACT AND CASTRATE C57BL/6J, DBA/2J, AND B6D2F1 MALE HOUSE MICE, S. D. Holman and R. E. Hutchison, MRC Neuroendocrine Development & Behaviour Group, IAPGR, Babraham, Cambridge, CB2 4AT, UK.

Aromatization of testosterone to estradiol is required for the differentiation of brain structure and behavior. In the dove and gerbil aromatase forms estrogen in preoptic cells associated with male sexual behavior. We compared the in vivo kinetic properties of the aromatase in the two species and found that expression and activity are not similar in the two species. We used as substrates. Adult dove aromatase is > 3 times more active as the developing (days 1-3 posthatch) POA, and has a Km 10 times lower than the developing enzyme (4 and 40 nM, respectively). Thus, there seem to be two isozymes of estrogen in the developing enzyme. With the different Km values, the active adult form is only expressed in later life. The kinetic of aromatase activity in the great gerbil does not show any sex dimorphism. Significant aromatic activity is also present in the neonatal cortex, but is absent from the adult. Preoptic aromatase activity in both developing and adult gerbil is much lower than in the dove. Using 3H-androstenol as substrate similar Km values were obtained for the aromatase within each species. We conclude that (a) there are species differences in the preoptic aromatase; (b) the substrate affinities of the avian enzyme and possibly its form changes during development. This change is not evident in the mammalian (gerbil) brain.

**561.8**

IN VITRO DEVELOPMENTAL CHANGES IN BRAIN AROMATASE OF THE DOVE AND GERBIL.


Aromatization of testosterone to estradiol is required for the differentiation of brain structure and behavior. In the dove and gerbil aromatase forms estrogen in preoptic cells associated with male sexual behavior. We compared the in vivo kinetic properties of the aromatase in the two species and found that expression and activity are not similar in the two species. We used as substrates. Adult dove aromatase is > 3 times more active as the developing (days 1-3 posthatch) POA, and has a Km 10 times lower than the developing enzyme (4 and 40 nM, respectively). Thus, there seem to be two isozymes of estrogen in the developing enzyme. With the different Km values, the active adult form is only expressed in later life. The kinetic of aromatase activity in the great gerbil does not show any sex dimorphism. Significant aromatic activity is also present in the neonatal cortex, but is absent from the adult. Preoptic aromatase activity in both developing and adult gerbil is much lower than in the dove. Using 3H-androstenol as substrate similar Km values were obtained for the aromatase within each species. We conclude that (a) there are species differences in the preoptic aromatase; (b) the substrate affinities of the avian enzyme and possibly its form changes during development. This change is not evident in the mammalian (gerbil) brain.

**561.9**

ARE BRAIN MECHANISMS OF SEXUAL BEHAVIOR IRREVERSIBLY LATERALIZED BY NEONATAL ANDROGENS IN GERBILS? S. D. Holman and J. B. Hutchison, MRC Neuroendocrine Development & Behaviour Group, IAPGR, Babraham, Cambridge, CB2 4AT, UK.

Androgens are linked to asymmetric brain development in the gerbil. The volume of the left, but not the right, sexually dimorphic area, pars compacta (SDA) is related to the rate of anterior hypothalamic aromatase (Holman and Hutchison, Dev. Brain Res., 1991). We compared the effects of neonatal castration (NC, n=8), laryngeal motor transection or sham -treatment on SDApc volume and vocalizations. Left and right SDApc volumes were symmetrical in the control non-lesioned group (left, right, rho=0.64, p<0.01). But the right SDApc volume was significantly reduced in the LD group (pretransect. = 561.7, posttransect. = 561.9). An age-related decline in reproductive, endocrine, and behavioral responses occurs in male Japanese quail. Decreased behavioral activity precedes measurable changes plasma testosterone and estradiol. Changes in hypothalamic catecholamines and reductase activity have been found during aging. This experiment investigates the immunoactivity (IR) of aromatase enzyme (AAR) in the preoptic area of young and aged male quail. Five young (6 months) sexually active and five old (3 yrs) quail whose androstenedione is > 3 times more active as the developing enzyme. (days 1-3 posthatch) POA, and has a Km 10 times lower than the developing enzyme (4 and 40 nM, respectively). Thus, there seem to be two isozymes of estrogen in the developing enzyme. With the different Km values, the active adult form is only expressed in later life. The kinetic of aromatase activity in the great gerbil does not show any sex dimorphism. Significant aromatic activity is also present in the neonatal cortex, but is absent from the adult. Preoptic aromatase activity in both developing and adult gerbil is much lower than in the dove. Using 3H-androstenol as substrate similar Km values were obtained for the aromatase within each species. We conclude that (a) there are species differences in the preoptic aromatase; (b) the substrate affinities of the avian enzyme and possibly its form changes during development. This change is not evident in the mammalian (gerbil) brain.

**561.10**

AGING AND PREOPTIC AREA AROMATASE IMMUNOREACTIVITY IN MALE JAPANESE QUAIL.

M. A. Ottinger, N. Thompson, T. Dellavalle, N. Harada* and E. T. Holman*, Univ. of Maryland, College Park, MD 20742, "Univ. of Virginia Charlottesville, VA 22903, and Fujita Health Univ. Toyoake, Japan.

An age-related decline in reproductive, endocrine, and behavioral responses occurs in male Japanese quail. Decreased behavioral activity precedes measurable changes plasma testosterone and estradiol. Changes in hypothalamic catecholamines and reductase activity have been found during aging. This experiment investigates the immunoactivity (IR) of aromatase enzyme (AAR) in the preoptic area of young and aged male quail. Five young (6 months) sexually active and five old (3 yrs) quail whose androstenedione is > 3 times more active as the developing enzyme. (days 1-3 posthatch) POA, and has a Km 10 times lower than the developing enzyme (4 and 40 nM, respectively). Thus, there seem to be two isozymes of estrogen in the developing enzyme. With the different Km values, the active adult form is only expressed in later life. The kinetic of aromatase activity in the great gerbil does not show any sex dimorphism. Significant aromatic activity is also present in the neonatal cortex, but is absent from the adult. Preoptic aromatase activity in both developing and adult gerbil is much lower than in the dove. Using 3H-androstenol as substrate similar Km values were obtained for the aromatase within each species. We conclude that (a) there are species differences in the preoptic aromatase; (b) the substrate affinities of the avian enzyme and possibly its form changes during development. This change is not evident in the mammalian (gerbil) brain.

**561.11**

ALZHEIMER'S DISEASE. R. Donahue, J.P. Ryan, and R.L. Heintz*. University of Ulm, D 7900 Ulm, Germany.

Glucocorticoids are assumed to exert differential effects on central nervous function through different receptor types described in rat brain (de Kloet & Reul, 1987). In a new experiment we studied the influences of hydrocortisone, which increases cortisol levels, as opposed to dexamethasone, which suppresses endogenous cortisol secretion, on sensory functions. In auditory tasks opposite effects of these steroids were most prominent on stapedial reflex: higher cortisol levels reflex needed more dB to be elicited. In the present experiment we studied this effect during the Morris water maze. In 24 subjects repeated measurements of stapedial reflex and salivary cortisol levels were carried out in parallel. Unexpectedly ss were asked to deliver a videotaped speech in bright light which led to a significant decrease in cortisol levels in most of the ss. A second control session followed the identical time course without speech. Significantly more dB were needed to elicit stapedial reflex at the time of cortisol maximum as compared to the minimum after recovery from stress 100 minutes later or to control session. Our results suggest that high glucocorticoid secretion attenuates perceptual abilities.

Quantitative receptor autoradiography is a powerful tool for studying steroid hormone activities. We report a precise quantification of radiolabeled hormone uptake combined with a high degree of anatomical resolution. Using immunohistochemical characterization of estrogen binding in the brain, it could only be achieved in vivo administration of radiolabeled estrogen prior to removal of the tissue samples for processing. We now report the application of an automated procedure which utilizes exchange labeling of occupied nuclear estrogen receptors by 125I in vitro. We then incubated with 2 nM 125I-labeled 11ß-methoxy-16α-o-iodoestradiol, a synergist of estradiol, for 1.8 or 27°C. The sections were washed, dried, and exposed to a sheet of high-resolution Hyperfilm for 24 hr. High levels of binding were evident in the medial and periventricular preoptic areas, bed nucleus of the stria terminals, ventromedial nucleus of the hypothalamus, the arcuate-median eminence region, and in the amygdala. Maximum binding was obtained at 2 hr and remained stable for up to 8 hr. Background levels were similar throughout the autoradiographic procedure and permits, for the first time, the selective autoradiographic analysis of occupied estrogen receptors in the brain. Supported by NRC Canada (N.J.M.) and NIH CA57799 (R.H.)

The brain as a source of circulating estrogen. B.A. Schlinger and A.P. Arnold. Department of Psychology and Brain Research Institute, UCLA, Los Angeles CA 90024-1563.

Local conversion of androgen to estrogen (aromatization) often precedes androgen action in the male brain. While the enzyme aromatase is ordinarily absent from the brain, it was found throughout the telencephalon of zebra finches, because E2 can also have demasculinizing effects in young females, and it is not clear that there are sex differences in plasma levels of estrogen during development. We have re-measured plasma levels of sex steroids during the first 13 days after hatching, and can detect no sex differences in levels of E2, estrone (E1), testosterone, androstenedione (A), or dihydrotestosterone (DHT). We also measured aromatase activity (1H-A conversion to 1H-E, and 1H-E2) in various tissues of hatchlings. Aromatase was abundant in ovary but was not detected in testes, adrenal, or other peripheral tissues of males. Aromatase was also found in the liver and testes, but there was no sex difference in homogenates of whole brain or in cellular fractions of telencephalon. The distribution of aromatase suggests that estrogen is the primary masculinizing agent during development of the vocal control system of males, androstenedione (A), or dihydrotestosterone (DHT). We also measured aromatase activity (1H-A conversion to 1H-E, and 1H-E2) in various tissues of hatchlings. Aromatase was abundant in ovary but was not detected in testes, adrenal, or other peripheral tissues of males. Aromatase was also found in the liver and testes, but there was no sex difference in homogenates of whole brain or in cellular fractions of telencephalon. Supported by NIH grants NS06469, DC00317, and NSF grant BNS9020953.

Castration alters tyrosine hydroxylase immunoreactivity in the medial amygdaloid nucleus of male hamsters. S.E. Asmus and S.W. Newman. Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

The medial amygdaloid nucleus (ME) processes chemosensory and hormonal information essential for mating behavior in the male Syrian hamster (Mesocricetus auratus). With colchicine pretreatment, the hamster ME contains a large population of tyrosine hydroxylase-immunoreactive (TH-IR) neurons, distributed preferentially in the midrostro and caudal regions of this nucleus. To determine whether TH production in these cells is influenced by gonadal steroids, the number of TH-IR neurons was compared in ME of castrated and intact hamsters. At 2, 4, and 12 weeks after castration, age-matched intact and castrated males were injected and compared for uptake with colchicine and perfused 48 hr later with 4% paraformaldehyde. Coronal brain sections (40 μm) were incubated with a monoclonal TH antibody (1:10,000; Incastar) which was visualized using the immunoperoxidase technique. The number of TH-IR neurons in midrostro ME declined to 48% of control values 4 weeks after castration. This number returned to control values 12 weeks after castration. The number of TH-IR neurons in caudal ME was similar in castrated and intact animals at all postcastration intervals. These results suggest that circulating testosterone maintains the levels of TH in midrostro ME, a region where lesions eliminate mating behavior. (Supported by NIH, NS 20629 to SWN.)

The brain as a source of circulating estrogen (aromatization) often precedes androgen action in the male brain. While the enzyme aromatase is ordinarily absent from the brain, it was found throughout the telencephalon of zebra finches, because E2 can also have demasculinizing effects in young females, and it is not clear that there are sex differences in plasma levels of estrogen during development. We have re-measured plasma levels of sex steroids during the first 13 days after hatching, and can detect no sex differences in levels of E2, estrone (E1), testosterone, androstenedione (A), or dihydrotestosterone (DHT). We also measured aromatase activity (1H-A conversion to 1H-E, and 1H-E2) in various tissues of hatchlings. Aromatase was abundant in ovary but was not detected in testes, adrenal, or other peripheral tissues of males. Aromatase was also found in the liver and testes, but there was no sex difference in homogenates of whole brain or in cellular fractions of telencephalon. Supported by NIH grants NS06469, DC00317, and NSF grant BNS9020953.

Neural distribution of estrogen receptor and LHRH mRNA in the male rat brain. L. S. Schlinger, R. K. GRIDLEY, and D. F. Read. Department of Psychology and Laboratory of Neuroendocrinology and Biological Timing, Univ. of Virginia, Charlottesville, VA 22901

Female musk shrews are sexually receptive shortly after weaning (21 days of age) and are fertile by 23 days of age. Also, we have detected a small number of estrogen receptors in the brain of these animals. The presence of estrogen receptors in the brain of the female musk shrew, and their potential role in mediating behavioral, as well as reproductive, responses to estrogen. Female musk shrews are sexually receptive shortly after weaning (21 days of age) and are fertile by 23 days of age. Also, we have detected a small number of estrogen receptors in the brain of these animals. The presence of estrogen receptors in the brain of the female musk shrew, and their potential role in mediating behavioral, as well as reproductive, responses to estrogen. Female musk shrews are sexually receptive shortly after weaning (21 days of age) and are fertile by 23 days of age. Also, we have detected a small number of estrogen receptors in the brain of these animals. The presence of estrogen receptors in the brain of the female musk shrew, and their potential role in mediating behavioral, as well as reproductive, responses to estrogen.
561.17

DISSOCIATION KINETICS OF NEURAL ESTROGEN RECEPTORS. Y. Chen* and N. C. Simon. Center for Molecular Bioscience and Biotechnology, Lehigh University, Bethlehem, PA 18015.

The biological potencies of a steroid-receptor complex may be linked to its dissociation rate (k_d) after transformation to a form capable of binding to DNA. Understanding this process may help elucidate a mechanism contributing to a particular target tissue sensitivity or insensitivity to a particular steroid. Our hypothesis is that a low dissociation rate is an essential determinant for sex-specific estrogen-dependent gene expression. We have transformed early dissociation rate data into dissociation kinetics. CF-1 female mice were maintained in accordance with Federal guidelines for animal care. Hypothalamic ER receptors were isolated and labeled with [3H]estradiol and [3H]testosterone. The dissociation kinetics were determined by the Scatchard method of transformed cells exposed to a constant steroid concentration and allowed to equilibrate in vitro. By binding estrogen-estrogen receptor (ER) complexes to hydroxyapatite (HAP) prior to activation, sufficient material was available to determine in vitro dissociation kinetics. CF-1 female mice were maintained in accordance with Federal guidelines for animal care. Hypothalamic ER receptors were isolated and labeled with [3H]estradiol and [3H]testosterone. The dissociation kinetics were determined by the Scatchard method of transformed cells exposed to a constant steroid concentration and allowed to equilibrate in vitro.

561.18


The medial preoptic area (MPOA) and medial amygdaloid nucleus (Me) are essential for male sexual behavior in the Syrian hamster. These nuclei integrate chemosensory stimuli and gonadal steroid signals, both of which are required for mating behavior. The objective of this study was to determine the distribution of androgen and estrogen-concentrating neurons in MPOA and Me in the male hamster using steroid autoradiography for estradiol (E2), testosterone (T) and dihydrotestosterone (DHT). Adult males (n=4 per group) received two injections of tritiated steroid 6-7 days after castration. The brains were removed and frozen 2h after the first injection, and 6 um frozen sections were mounted onto NTB-2 emulsion-coated slides. The slides were developed with D-19 after exposure for 11-16 months. In both MPOA and Me, neurons were more abundant and more heavily labeled with E2 treatment than with either T or DHT. Estradiol and DHT-labeled cells were scattered throughout Me, with a high concentration in posterodorsal Me. T-labeled cells were confined to posterodorsal Me. In MPOA, the majority of E2 and DHT-labeled neurons were in the medial preoptic nucleus (MPN) and the preoptic regions of the medial bed nucleus of the stria terminals. T-labeled cells in MPOA were most prevalent in the MPN. Throughout Me and MPOA, the distribution of E2-labeled cells is more widespread than that of T or DHT. These results provide a detailed comparison of E2, T, and DHT-concentrating neurons in nuclei important to the control of mating behavior. (Supported by NIH-NS-20629)

561.19


In order to investigate the mechanisms by which gonadal steroids regulate plasticity in the avian song control circuit, we cloned the canary androgen receptor (CAR) and have begun experiments to examine its localization. By in situ hybridization using single-stranded 32P-labeled RNA probes, the receptor message can be detected in the brain but is of very low abundance. The CAR mRNA appears to be relatively enriched in several of the functionally defined song nuclei of the canary brain, including HVc, RA and MAN, which concentrate labeled testosterone (Ardoin, JCN 189-421, 1980). Receptor expression in these structures is consistent with peripheral ER studied in a free receptor assay. The k_d for the two components were 5.1 ± 2.3 x 10^-3 min^-1 and 6.1 ± 1.5 x 10^-3 min^-1. Salt concentration (0.04M or 0.4M KC1) did not significantly affect the dissociation rate of either the fast or slow component, although the 0.4M KC1 condition slightly increased the proportion of activated ER.

561.20

ESTROGEN-RECEPTOR IMMUNOREACTIVE NEURONS IN THE FOREBRAIN OF ADULT MALE AND FEMALE FERRETS. S.A. Tobet, M. Basham*, T.O. Fox, & M.J. Baum. Dept. of Biochem., EK Shriver Ctr., Waltham, MA 02254 & Prog. Neurosci., Harvard Medical School, Boston, MA 02115, & Dept. of Biology, Boston University, Boston, MA 02215.

We have previously described estrogen binding neurons in and around the preoptic/anterior hypothalamic area (POA/AH) of male and female adult ferrets using autoradiographic methods (Tobet & Baum, 1991; Dev Brain Res, in press). The present study extends our observations to estrogen receptor immunoreactive neurons throughout the basal forebrain in the context of regional selectivity and sex differences. Estrogen receptor immunoreactivity was visualized using the H222 estrogen receptor antibody (Abbott Laboratories) and Nickel/DAB reaction product (H222Ir). One of the most striking features of H222Ir cells was the presence of H222Ir in neuronal processes. All cells with H222Ir in processes also contained H222Ir in nuclei, although the converse was not true. In females, increased estradiol levels were associated with decreased immunoreactivity in processes. Cells in the bed nucleus of the stria terminus and ventral lateral septum were notable for the absence of H222Ir in processes. The lateral hypothalamic was rich in H222Ir processes in both sexes. More H222Ir was noted in the processes of female cells in the dorsal lateral septum and medial POA/AH and at the level of the descending fornix and caudal anterior commissure. The H222Ir in neuronal processes in the region comparable to the MN-POA/AH in females presented the appearance of nuclear grouping even though no nucleus is apparent by Nissl stains. The differential dendritic organization of estrogen-binding in multiple brain regions may provide another level of regulation for sex-selective estrogen-dependent functions.

562.2 DO SEXUALLY MONOMORPHIC PATTERNS OF PLASMA E2 AND ANDROGENS DURING NESTLING DEVELOPMENT ACCOUNT FOR THE LACK OF A SEX DIFFERENCE IN ADULT SINGING BEHAVIOR IN THE EUROPEAN ROBIN? E.S. Hiatt and H. Schwabl*. Max-Planck-Institut für Verhaltensphysiologie, Andecks, D-8133, W. Germany; Dept. of Biology, Northeastern Univ., Boston, MA 02130 and Rockefeller Univ. Field Research Center, Millbrook, NY 12545.

45 Circulating levels of estradiol-17β (E2), aromatizable testosterone (T) and non-aromatizable 5α-dihydrotestosterone (DHT) were measured in male and female European robin chicks from day 3 to day 19 after hatching. E2 concentrations were significantly lower in males than in females, but varied significantly with age in males. Levels in males were higher during days 13 than days 11 and 19, when they were close to basal (50 pg/ml in our RIA system). Levels of T were low though not basal during the first days after hatching in both sexes. They were high at the time of fledging (day 13-15) and decreased by day 19 to basal (60-100 pg/ml). No sexual dimorphism in T levels was apparent. Levels of DHT were very high in both sexes during the early nestling phase and then steadily decreased to basal levels by day 19 in both sexes. These results are consistent with the hypothesis that in species of songbirds in which both sexes sing as adults, as in the robin, levels of sex steroid hormones are elevated and sexually monomorphic during early development, when the neural song control system is organized.

562.3 N-METHYL ACETIC ACID INJECTIONS INTO THE MEDIAL AMYGDALA FACILITATE MATERNAL BEHAVIOR IN RATS. M. Barba, M. Linster* and J.B. Englehart. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

The inhibitory role of the medial amygdala (MA) in maternal behavior control was explored. Injections of neurotoxic doses of N-methyl acetic acid (AMA: 8 μg/ul) into MA facilitated maternal behavior in nulliparous female rats when pups were added to them 12 days following the injections. This effect was specific to MA in that AMA injections into the basolateral amygdala were ineffective. Also, the facilitatory effect of AMA injection into the MA was eliminated when ovariotomies were performed at the time of brain surgery. In a subsequent experiment, vaginal smear data indicated that AMA-MA injections induced a pseudopregnant state that lasted about 13 days in females that retained their ovaries. That this pseudopregnant state contributed to the facilitation of maternal behavior induced by AMA-MA injections. A final experiment is testing this view further by presenting test pups to females 24 days, rather than 12 days, after AMA-MA injections. It appeared that prolonged AMA-MA underlies the observed facilitation of maternal behavior.

Supported by a Whitehall Foundation grant.

562.4 COCAINE DISRUPTS MATERNAL BEHAVIOR IN LACTATING RATS. C.H. Kinsley, A. Bauer*, M. Beverly, D. Turco*, J. Wellman. Department of Psychology, University of Richmond, VA 23173.

Though much attention has been devoted to the behavioral and physiological consequences of cocaine (COC) abuse, little is known regarding the effects on maternal behavior (MB). We examined whether COC affects the initiation (late pregnancy) and/or maintenance (postpartum [PP]) phases of MB. In Exp. 1 COC (5 or 10 mg/kg) or saline was administered on PP day 5 and/or 6 and MB scored. 5 mg/kg was marginally effective (p<.05), whereas 10 mg/kg significantly disrupted MB. Exp. 2 examined specific elements of the disorganization, and found increases in the latencies to contact, retrieve, lick, group and crouch over pups. In Exp. 3 osmotic minipump (P) controls with either saline (S), progesterone (P), or empty Silastic capsules. There was a trend toward larger pups with significantly lower body fat contents in hams ters treated with E and with Exp. Taken together, the above results suggest that cocaine increases the metabolism of Syrian hams ters under environmental and reproductive conditions in which energy demand is high and energy availability low. (Supported by NS10873, AM30976 from NIH, and BNSR71961 from NSF.)

Precipitated we demonstrated that bilateral lesions of the habenular complex (Hbc) made on day 12 of pregnancy severely disrupt the hormonal onset of maternal behavior (MB) (retrieving, nursing and nestbuilding) in the rat for 8-10 days. However, by day 14 (3 days after the lesion) the majority of animals in the Hbc lesion group displayed complete MB compared with unoperated controls. These results suggested two hypotheses: either there was a recovery by the 14th following the lesion, or pup exposure for 8d stimulated MB despite bilateral lesion. An additional hypothesis was made: Bilateral Hbc lesions were made 4 days earlier on day 8 of pregnancy to determine if the passage of time (13d) following the lesion was sufficient for behavioral recovery. All animals were hysterectomized, and given 20mg/kg estradiol benzoate on day 16 of pregnancy, then tested 4h later with pups for MB. The majority of Hbc-lesioned animals show complete MB by day 3 of testing. Thus, Hbc lesions made earlier in pregnancy result in an earlier recovery in MB. These data suggest that a neural recovery process requiring approximately 13 days may account for the behavioral recovery seen after Hbc lesions. (HD2983 to J.I.M.)


Seasonally breeding adult male rhesus monkeys (n=8) were moved from outdoor breeding groups to a light-controlled (16L:8D) indoor environment on June 21 (mid non mating season) and remained on this lighting schedule for 18 months. At the onset of the following mating season, males were randomly divided into two groups and underwent 4 phases of 17 weeks of Melatonin (50mg Silastic capsules producing 70-80pg/ml serum melatonin) or Control (empty capsule) treatment. For group A, the sequence of control (C) and melatonin (M) treatments in the 4 phases of the study was CMCM; for group B, the sequence was CCMC. The reproductive status of untreated outdoor-housed rhesus monkeys during the four 17-week phases was: (1) early mating season, (2) late mating season - early non-mating season, (3) late mating season - non-mating season, and (4) early mating season in both groups, phases 1, 2, and 4 were characterized respectively by increasing, decreasing and increasing mounting frequency, testosterone level and testis volume. This is similar to the seasonal pattern shown by outdoor-housed animals, and likely represents persistence of an endogenous reproductive rhythm. During phase 3 (when outdoor-housed animals showed no sexual behavior), the group receiving melatonin (group B: CCMC) displayed significant increases in frequencies of mounting. Males not receiving melatonin during phase 3 (group A: CMCM) mounted at a significantly lower rate. Testosterone levels and testis size in the two groups changed in the opposite direction with melatonin treatment, and likely represents persistence of an endogenous reproductive rhythm. This poster will report attempts to associate changes in beta-endorphin-like immunoreactivity, cortisol, prolactin and progesterone. During analysis, six unique patterns of PS were noted. This poster will report attempts to associate hormone levels and patterns with the more rigorous symptom pattern categories.


During selective breeding for degree of lateralization for handness in mice, an interaction of parity and sex emerged for the direction of asymmetry of selected mouse lines. This interaction affected quantitative and qualitative measures of laterality in males of selected mouse lines: Pr(RH|L1) = 0.399; Pr(RH|L2) = 0.601; Pr(RH|G8) = 0.371; Pr(RH|G8) = 0.406 and 0.490, respectively; p = 0.00004. No interaction affected quantitative and qualitative measures of laterality in females of selected mouse lines. An interaction affected quantitative and qualitative measures of laterality in males of selected mouse lines: Pr(RH|L1) = 0.434; Pr(RH|L1) = 0.490 and 0.490, respectively; p = 0.00004. No interaction affected quantitative and qualitative measures of laterality in females of selected mouse lines.

562.8 HORMONAL AND ENVIRONMENTAL INFLUENCES ON MALE INCUBATION IN SEX-ROLE REVERSED SHOREBIRDS. A.V. Fnavizani and L. Halanana. *Biology Dept., Univ. of North Dakota, Grand Forks, North Dakota 58202.

In a few avian species such as the spotted sandpiper and Wilson's phalarope, males provide all or most of the parental care in breeding during the early reproductive years. Plasma levels of prolactin, the hormone most associated with parental care in birds, is sex-reversed being higher for males than for females. Plasma testosterone declines rapidly at incubation and experimentally maintained high levels inhibit normal incubation behavior. Administration of the androgen blocking agent flutamide, facilitates the onset of incubation. Experimentally switching eggs of known incubation duration to produce longer or shorter than normal incubation periods for particular males indicates that the presence of eggs maintains prolactin beyond the normal time of hatch and the presence of chicks or absence of eggs results in the decline of prolactin before the normal time of hatch. Male incubation appears to be correlated with elevated prolactin, low testosterone and the continued stimulus of eggs in the nest.

562.10 DIFFERENTIATION OF PERIMENSTRUAL SYMPTOM CATEGORIES AND ENDORCINE CORRELATES. C.A. Cahill. University of Kansas Medical Center, School of Nursing and School of Medicine Kansas City, KS 66103.

The etiology of dysphoria associated with the last week of the menstrual cycle has puzzled many. Attempts to associate changes in gonadal hormones with symptom severity and type have not clearly cut associated symptoms. Several hypotheses have been offered to explain this apparent contradiction. One possibility is that several different mechanisms are associated with Perimenstrual Symptoms (PS) which may be reflected in different types of symptoms, symptom severity and symptom patterns. In this study, 40 women were selected for study based on symptom type and severity. Twenty women with minimal symptoms and twenty with moderate to severe depression-like symptoms were studied over three consecutive menstrual cycles. Plasma samples were collected twice per week and analyzed for beta-endorphin-like immunoreactivity, cortisol, prolactin and progesterone. During data analysis, six unique patterns of PS were noted. This poster will report attempts to associate hormone levels and patterns with the more rigorous symptom pattern categories.


A multivariate assessment of the spontaneous locomotor activity of the laboratory bred Mongolian gerbil (Meriones unguiculatus) was conducted by using a Digital System. Spontaneous locomotor activity data were collected for 12 consecutive 5 min. samples from each of 42 gerbils obtained between 4 and 97 days of age (24, 39, 62, 116, 150, or 197 days). Variables included examined: total distance travelled (TD), average distance (AD), average speed (AS), number of horizontal movements (NMH), number of vertical movements (NMV), time per horizontal movement (TMT), number of vertical movements (NVV), time per vertical movement (VTM). Results indicated that across the 12 samples, all activity measures (except AS and TMT) declined for both males and females. Overall differences in locomotor activity between males and females were found over the one hour period on the measures AD and AS. Females exhibited higher levels of activity. Sexual differences on the measures TD, NV, and VT were strongly reduced after approximately 30 minutes. Age also had an effect on spontaneous activity. All measures became significantly greater for the early sex ages (i.e. for the litters aged 24, 39, and 62 days), and remained relatively constant thereafter (i.e. for the litters aged 116, 150, and 197 days). The activity levels over the one hour period declined more rapidly for the younger litters on the measures of TD, AD, NM, and MT. These results extend previous work examining sexual differences in laboratory rats and mouse voles using the Digital System.

Several lines of evidence suggest that vasoactive intestinal polypeptide (VIP) is an important factor in birds. Systemic VIP increases, while antibodies to VIP decrease plasma prolactin (PRL). Additionally, VIP decreases PRL in dispersed pituitary cells in vitro. Although VIP-like immunoreactive cells are widely distributed in the avian hypothalamus, one group in the infundibulum is known to be increased during incubation.

To assess whether PRL influences hypothalamic VIP, the effect of ovine VIP (o-VIP) was investigated. Breeding does were given 6 daily injections of o-VIP beginning on incubation day 8 (I 8), while controls received vehicle. Body weight and behavior were measured during incubation, revealing that during periods of increasing body weight, on I 4, crops weights and gonadal states were measured. The brains were removed, sectioned, and alternate sections were stained for VIP and gonadotropin releasing hormone (GnRH).

PRL decreased detectable VIP-like immunoreactive cell number in the IPF, but did not change lateral septal VIP or mediod preoptic (MPOA) GnRH cell number. O-PRL treated subjects had lower crop weights than I 4, but did not differ from controls in behavior or gonadal state. The results suggest that during incubation, central PRL regulates its own secretion by acting on IPF VIP cells. Moreover, the site of feedback is limited to the population of VIP cells that increase in diameter during incubation. Supported by NIMH grant 29380-15.


Previous work from our laboratory has shown that when 6-day-old rats are stroked on the sides, flanks, and rump they display a lordotic-like response by pressing with extracellular benzodiazepine (BDZ) and progesterone (P). In adult female rats, it is believed that cutaneous input from the anterior curve of the hip is the critical sensory input for the lordosis response. Since progesterone and gonadectomy females show fast extinction rates, would it be expected that females, males, and adult rats would show fast extinction rates. Deprived intact males also exhibit fast extinction rates. The following two studies were designed to determine the relationships between T and P in Fischer 344 males. In both studies, a CTA was induced in males under a 23 hr FD schedule or under no deprivation (ND) control conditions. No males show fast extinction rates. Deprived intact males also show fast extinction rates. The following two studies were designed to determine the relationships between T and P in Fischer 344 males. In both studies, a CTA was induced in males under a 23 hr FD schedule or under no deprivation (ND) control conditions. No males show fast extinction rates.

During acquisition but not extinction, T acts during acquisition but prolongs extinction. These results can be accounted for by anxiolytic properties of P. Physiological levels of systemically injected progesterone (P) have been shown to amplify GABA responses of neurons within a model CNS circuit (Smith et al., 1987a,b,c; 1989) in a manner similar to the benzodiazepines (BDZ), an effect due to benzodiazepine receptor interaction.
563.5

SITE-SPECIFIC INJECTIONS OF A SELECTIVE OXYTOCIN RECEPTOR ANTAGONIST AFFECTS FEMALE SEXUAL BEHAVIOR

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In this study we used mice, genetically selected for aggression. At adult age aggressive males, with a Short Attack Latency (SAL), possess higher plasma testosterone (T) level compared to males with a Long Attack Latency (LAL). In contrast, at neonatal age, in LAL males a higher T production occurs. In the adult brain, the vasopressinergic (AVP) innervation of the lateral septum (LS) is less abundant in these SAL males. Probably, the neonatal difference in plasma-T level is involved in a differential development of the AVP system in these two selection lines. In order to establish whether the laterality of this system is involved in the differentiation in aggressive behaviour. Therefore adult T treated SAL and LAL males were castrated, and aggression and AVP content were measured 15 weeks later. We found an almost complete reduction in LS innervation of AVP in both selection lines. No difference was found in the nucleus of the solitary tract (NTS), however, a higher plasma AVP level was measured in the T treated LAL males than in T treated SAL males. Surprisingly, only the adult LAL males show a reduction in aggression. Thus, although AVP is T dependent in both selection lines, only the LAL males are affected in aggression after castration. This suggests a differential involvement of T dependent AVP systems in the organization of aggressive behaviour in the two selection lines of mice.

The investigation was supported by the Foundation for Biological Research (BION-426.06), which is subsidized by the Netherlands Organization for Scientific Research.

563.6

ACTIVATION OF C-FOS-LIKE PROTEIN IN PVN OXYTOCIN CELLS DURING MALE SEXUAL BEHAVIOR


The protein product of the c-fos oncogene has been previously used to map neuronal activity at the cellular level following seizures, dehydration, stress, and a variety of drug treatments. In the current study, induction of c-fos-like protein was used to immunocytochemically follow male sexual behavior to determine neuronal sites of activation associated with mounting and ejaculation. Sexually experienced male rats were divided into four groups of rats: (a) ejaculation (ejaculated males); (b) intromission and mounting without ejaculation; or (c) exposure to a testing arena without any prior estrous female. Induction of c-fos-like protein was not observed in sexually naive rats following any of the treatments, although this area has been previously implicated in the mediation of male sex behavior. Treatment effects were noted in the parvocellular and magnocellular divisions of the hypothalamic area of the PVN with increases of 300-500% in the number of parvocellular neurons expressing c-fos following either mounting or ejaculation. Significant but less striking increases were noted in the parvocellular area of the PVN with increases of 250-300% in the number of parvocellular neurons expressing c-fos following copulation. Ongoing experiments are investigating other neuropeptide phenotypes. These results are generally consistent with other techniques implicating extra-hypothalamic oxytocin projections in the mediation of male sex behavior. The absence of c-fos induction in the MPOA does not rule out the possibility that some other transcription factor may be associated with cellular activation in this region.
563.9 HOMOZYGOUS BRATTLEBORE RATS DISPLAY ATTENUATED CONDITIONED FREEZING RESPONSES. James D. Stope*, Savio Cheng*, Frances McCormack and William G. North, Dept. of Physiology and Biophysics, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814.
Rats homozygous for hereditary hypophalamic diabetes insipidus (Brattlebore rats) lack measurable levels of AVP in the CNS and peripheral circulation, while rats heterozygous for this condition have a reduced capacity to synthesize AVP that results in levels that are less than half of those found in normal Long-Erectus (LE) rats. In the present study we examined the influence of AVP on conditioned freezing behavior by comparing the responses of Brattlebore homozygous (DI) rats, Brattlebore heterozygous (HZ) rats, and LE rats. Animals were placed in a sound-attenuated shock chamber on the training day and given a series of 3 footshocks (1 ma, 0.75 sec, 20 sec inter-shock interval). On the following four days the rats were placed in the chambers where they had received their shock and levels of spontaneous freezing evaluated.
For each of the four days, DI rats displayed significantly less freezing behavior when compared with LE rats and HZ rats. HZ rats displayed trends towards attenuated freezing compared when compared with LE rats. The data then imply that, in rats, a correlation exists between the capacity to produce AVP, and the amount of freezing displayed. These preliminary results suggest that vasopressin may play a significant role in the appropriate autonomic and emotional responses to fearful stimuli.

Facilitation of female sexual receptivity is a complex behavior regulated by neuropeptides. Oxytocin (OT) facilitates lordosis, pelvic, hypogastric and combined neurones were performed. Ovariectomized rats received 2.5g EB for 3 days and 500g P, four hours prior to behavioral testing. Ten minutes before testing, females received either 0.1cc saline or 2.0ug oxytocin. They were then placed in a P-ill all with a sexually experienced male. Males were allowed to deliver 10 mounts or intromissions and the IQ for each female was determined. One week later, the test was repeated and the drug treatments were reversed. Animals then received either bilateral pelvic, hypogastric or combined neurones. After recovery, animals were tested as described. The results indicate that pelvic and combined neurones may have a significantly greater effect on reducing oxytocin-induced facilitation of behavior than hypogastric neurones. This suggests that the pelvic nerve is more important than the hypogastric nerve in facilitating sexual receptivity.

563.11 VASOPRESSIN V1a RECEPTOR BINDING IN RAT AND HAMSTER ESTROUS CYCLE: EFFECT OF Gonadal STEROIDS. A.E. Johnson, S. Audigier*, J.H. Gough*, S. Just*, L.E. Allen, Karolinska Institute, Clin. Res. Ctr., Huddinge Hosp., Huddinge, Sweden; Centre CNRS-INSERM de Pharmacologie-Endocrinologie, Monpellier, France; Dept. of Biology and Psychology, Lab. of Neuroendocrinology and Behavior, Georgia State University, Atlanta, GA.
In rats and hamsters, pregnancy (P) vasopressin (VP) vasopressin receptor binding is involved in the regulation of a variety of social behaviors that are dependent on circulating gonadal hormones. In rats, uterine steroids modulate VP transmission by altering the synthesis or function of certain brain receptors that do not appear to regulate central VP receptor binding. In hamsters gonadal steroids do not affect VP peptide levels, however their effect on central VP receptors are unknown. To further investigate the role of VP transmission in the expression of steroid-dependent behavior, the distribution and central VP-receptors by steroide steroids was studied in rats and hamsters using the highly selective V1a-receptor ligand, [125I]-labeled D-Tyr(8)-DesGly(9,10)-[D-Tyr(8)]-Oxytocin (125I)-OK. Side mounted tissue sections were preincubated in 50mM Tris buffer (pH 7.4) followed by a 1 hr incubation (20°C) in buffer containing 50mM Tris-150 NaCl 1% albumin, 5mM MgCl2, 0.1mM BSA. The incubation was terminated by washing the sections in ice-cold buffer containing 150 NaCl 1% BSA. The sections were then dried and exposed to film. The results indicate that pelvic and combined neurones may have a significantly greater effect on reducing oxytocin-induced facilitation of behavior than hypogastric neurones. This suggests that the pelvic nerve is more important than the hypogastric nerve in facilitating sexual receptivity.

Recent evidence suggests that the parietal hormone oxytocin (OT) acts as a neurotransmitter in the brain to modulate female sexual behavior. Earlier reports from this laboratory demonstrated that OT receptor binding in the VMN is induced by estrogen treatment, and that progesterone causes this binding to spread laterally. In the present series of experiments the OT innervation of the VMN was examined. Using immunocytochemical techniques, oxytocin fiber levels in the VMN were compared in ovariectomized rats treated with oil or estradiol (10 μg in 100 μl for 2 days, sc). The number of OT fibers in the VMN was small, whereas the number of oxytocin fibers were increased prior to the estrus period. At this time, the parietal was relatively small. No differences were observed in the OT fibers within the VMN in the oil-treated versus the estradiol-treated group (n=9 per group). In a second experiment, the retrograde tracer Fluoro-Gold (FG) was injected into the VMN of female rats. Seven days later the rats were perfused and OT neurons were stained with the immunofluorescent marker Texas Red. Co-localization of FG with FG occurred only occasionally when FG injections were confined to the VMN of female rats. The results indicate that OT neurons do not innervate the VMN itself but rather VMN dendrites that extend laterally towards the OT fiber tract. Thus, the progesterone-induced movement of OT receptors may be the key factor responsible for the increase in OT fiber density in the anterior hypothalamus. The results of this study suggest that the parietal OT neurons innervate the VMN and the lateral parietal VMN in ovariectomized rats. Supported by NIH grant NS07080 and NIMH postdoctoral training grant MH11525.

Microinjection of arginine vasopressin (AVP) or vasopressin into the lateral septum and/or anterior hypothalamus (AH) stimulates flank marking, a stereotypic motor behavior in hamsters. The present results were undertaken to determine which of these two sites is more critical in the organization and expression of AVP-dependent flank marking. Since the LS and the AH have unilateral and reciprocal connections, AVP was injected into the LS in animals with neotenic lesions in the ipsilateral AH or AVP was injected into the AH in animals with ipsilateral lesions in the LS. Lesions in the LS blocked flank marking only when the LS was also lesioned. Lesions in the AH blocked flank marking triggered from the LS but not vice versa. The efficacy of the neotenic lesions was assessed by the loss of AVP receptor binding following in vitro autoradiography. Furthermore, unilateral lesions of the AH also blocked flank marking stimulated by AVP injections in the contralateral LS, suggesting that flank marking requires the recruitment of neurons from both sides of the AH. The following data support this notion. First, unilateral lesions of the AH block flank marking induced by AVP injection in the contralateral AH. Second, unilateral injection of AVP into the AH stimulates comparable c-fos immunolabelling within both sides of the AH, suggesting the existence of an unknown site providing bilateral recruitment of the AH for the expression of flank marking.
563.19
CRF ALTERS MONOAMINE AND MONOAMINE METABOLITE LEVELS IN DISCRETE MOTOR AREAS IN AMPHIBIAN BRAIN. C.A. Lowry, K.J. Reiner, L.M. Laughlin* and F.L. Mosser*. Dept. of Zoology, Oregon State University, Corvallis, OR 97331-2914.

Studies using mammals and the amphibian Tariche granulosa indicate that CRF may elicit neuroendocrine, autonomic, and behavioral events following exposure to stressful stimuli. Included among the behavioral responses to CRF is an increase in locomotor activity. Evidence suggests that in rodents CRF-induced locomotor activity may be mediated by monoamine systems. The present study investigated in T. granulosa the effects of intracerebroventricular injections of CRF on monoamine and monoamine metabolite levels in discrete brain areas.

We characterized the distribution of epinephrine, norepinephrine, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) using brain microdissection and HPLC with electrochemical detection. No injection of CRF (150 pmol) into the periventricular organ (PVN) induced changes in DA or 5-HT metabolite levels in the T. granulosa brain. The injection that did alter monoamine levels was 50 pmol of CRF injected into the ventral striatum (VSt). Compared to control animals, CRF-injected animals displayed increased locomotor activity in an open field and had altered levels of monoamines or monoamine metabolites in two brain areas, the ventral striatum and the PVN. CRF-induced locomotor activity is associated with changes in the 5-HIAA/5-HT ratios in the ventral striatum and with DA and 5-HT levels in the periventricular organ. Supported by NSF BNS-9009173.

564.1
EFFECT OF (+)-MK-801 AND KETAMINE ON RAPID TOLERANCE, CROSS-TOLERANCE AND CHRONIC TOLERANCE. J.M. Khanna, J. Heling*, P.H. Wu* and H. Kalant*. University of Toronto, Toronto, Canada MSS 1A8 and Addiction Research Foundation, Toronto, Canada M5S 2S1

Hypophagia and motor-impairment (tilt-plane test) were used to assess whether N-methyl-D-aspartate (NMDA) receptors play a role in the development of rapid tolerance to ethanol, i.e., tolerance to a second dose of ethanol given 24 hr after the first dose. Results showed that (+)-MK-801 and ketamine blocked the development of rapid tolerance to ethanol on both tests. Similarly, both NMDA antagonists blocked cross-tolerance from ethanol to chloridazepoxide as well as from chloridazepoxide to ethanol on both tests. These data suggest that the role of NMDA receptors in ethanol tolerance may be similar to their role in memory and learning, involving a facilitation of transmission in certain synapses. In contrast, both NMDA antagonists failed to block chronic tolerance to ethanol in either test. They did not modify blood alcohol levels in any of the groups, so the blockade of rapid tolerance cannot be attributed to changes in pharmacokinetics of ethanol. It is possible that NMDA antagonists lose their effectiveness with chronic administration or that the phenomena of rapid tolerance and chronic tolerance have basic differences not previously reported. Supported in part by NIAAA grant No. 1 R01 AA02112-02.

564.2
CHRONIC ETHANOL TREATMENT ALTERS SENSITIVITY TO ACETYLCOLINE IN RAT HIPPOCAMPUS. B.S. Ruberg and B.E. Hunter, Department of Neuroscience, University of Florida, Gainesville, FL 32610.

Sensitivity of hippocampal field potentials to local (iontophoretic) application of acetylcholine (ACh) was investigated in chronic ethanol treated (CET) and sucrose-fed (control) rats. CET and control rats were fed a liquid diet containing either ethanol or sucrose for 28 weeks. Five to six months after ethanol or sucrose was withdrawn, hippocampal slices were taken and ACh was applied in stratum pyramidale or stratum radiatum of CA1 to observe population spike facilitation or field EPSP inhibition, respectively. An antidiromic-orthodromic paired pulse paradigm (PPP) was also used to assess interactions with recurrent inhibition.

Population spines were facilitated to a considerably lesser extent in CET slices relative to controls, while no treatment differences were observed for dendritic EPSP inhibition. Although preliminary, PPP data suggested that ACh interactions with inhibitory circuitry were also affected. These results suggest a heterogeneous effect within CA1 to the chronic actions of ethanol. Since these cholinergic responses have been linked to differing muscarinic receptors, our results may reflect a distinct susceptibility of muscarinic receptor subtypes to the neurotoxic effects of ethanol. (Supported by the Veterans Administration and NIAAA #AA00200)

564.3

Corticotropin-releasing factor (CRF), acting as a neurotransmitter in both hypothalamic and extrahypothalamic areas, is involved in mediating the endocrine, autonomic and behavioral responses to stress. Short- and long-term exposure to alcohol has been demonstrated to activate the hypothalamic-pituitary-adrenal (HPA) axis in both humans and laboratory animals. Because many of the signs and symptoms observed in animals and humans following ethanol withdrawal resemble those of stress, we investigated the effect of chronic ethanol administration (28 days) followed by abrupt ethanol withdrawal on hippocampal and extrahypothalamic CRF concentrations, and CRF receptor number and affinity. CRF concentrations were not altered by chronic ethanol administration or withdrawal in any of the thirteen brain regions studied. CRF receptor number was significantly increased in the prefrontal cortex, piriform cortex and cerebellum following chronic ethanol administration. CRF receptor number was decreased in the anterior pituitary following chronic treatment and abrupt withdrawal, indicative of down-regulation of CRF receptors due to increased CRF release into the hypophysial portal vessels. The present results indicate that ethanol exerts effects on extrahypothalamic CRF systems. Supported by NIMH MH-42088.

564.4

The effect of chronic alcohol exposure and withdrawal from this exposure has been studied in the alcohol-sensitive line (SS) and alcohol-insensitive line (LS) of the glass frog, Rana pipiens (30 mice total). The mice were fed for 4 months either with a control isocaloric liquid diet or with an isocaloric liquid diet containing ethanol (23.3% ethanol derived calories). Some of the ethanol treated mice were withdrawn from the diet for 1 month. Subsequently the mice were prepared for electron microscopy and the GABA terminals were labeled with antiGABA antibodies using a gold probe in a postembedding protocol. GABA terminals make synaptic contacts either on dendrites or dendritic spines of the dentate granule cells. The number of axodendritic and axospinous synapses were counted separately for the proximal third and distal two-thirds of the dentate molecular layer. A gradual loss of axodendritic synapses was seen in the LS mice. A 14.5% loss (statistically nonsignificant) of axodendritic synapses occurred after 4 months of ethanol exposure. This loss continued during the withdrawal period resulting in 38% fewer axodendritic synapses (p = 0.005). No changes were observed in the number of axospinous synapses. No loss in either type of contacts was seen in the SS line following ethanol exposure and withdrawal. However, when LS and SS controls were compared, significantly fewer axodendritic synapses (by 29%, p = 0.033) were observed in the SS line. These observations are in line with our previous findings showing a loss of GABAergic basket cells in LS mice following chronic alcohol exposure. This observation could be interpreted as an adaptation of the nervous system to the depleting effect of ethanol. Interestingly, the change starts during ethanol exposure and gets more severe during ethanol withdrawal. Supported by NIAAA grant #AA000200 and NIMH grant #MH48134.
564.4 CHRONIC INTERMITTENT ETHANOL IN RATS ALTERS \(^{[7]}\)HRO15-4513 BINDING TO DIAZEPAM-INSENSITIVE GABA-RECEPTORS. D.M. Sapp, S.K. Kokke, and N.W. D'Amico, Dept. of Pharmacology, Brain Research, University of California, Los Angeles, CA 90024.

Chronic intermittent ethanol (CIE) administered to rats produces a 'kindling-like' phenomenon in which the PTZ-induced seizure threshold is decreased. A change in PTZ-induced seizures sensitivity suggests that the GABA receptor complex is involved in the differences observed between control and CIE animals. GABA receptor binding was compared between the two groups of animals using the partial inverse-agonist \(^{[7]}\)HRO15-4513. This ligand was used because of its reported 'alcohol antagonist' activity and its unique binding to both diazepam-sensitive (DZ-S) and diazepam-insensitive (DZ-IS) sites. Crude membrane homogenates were prepared from both the cortex and cerebellum. Several differences in binding were observed between the two groups of animals. In the cerebellum of CIE animals, the binding of RO15-4513 to DZ-S sites is increased while the binding to DZ-IS sites is decreased, i.e., the ratio of DZ-S is decreased. The PET analysis indicates that the affinity of RO15-4513 for the DZ-IS sites is decreased two-fold. The ability of diazepam to displace RO15-4513 binding is also increased in CIE animals. Chronic ethanol thus produces a change in the properties or amount of DZ-IS binding sites for RO15-4513, possibly mediated by a switch in subunit gene expression.


Northern hybridization studies using a \(32P\)-c-fos murine DNA probe revealed that peak activation of rat brain cells leading to expression of c-fos mRNA occurs at 6-8 hr after the initiation of withdrawal from a 7 day continuous exposure to ethanol vapor. This experimentally induced block was followed by a 4 hr pretreatment with the NMDA receptor-associated ion channel blocker, MK-801 (10 mg/kg, i.p.). During this period of withdrawal, the concentration of glutamate in the dialysate from a 2 mm microdialysis probe implanted into rat striatum under chloral hydrate anesthesia was estimated by HPLC to be 120 pmol/30 ul vol/20 min. This was a significant (p<0.01) fold increase over the control value of 30 pmol/30 ul vol/20 min. No difference was seen in the levels of GABA, glycine, taurine or other detected amino acids. The data suggest a role for glutamate and NMDA receptors during ethanol withdrawal.

564.6 CHRONIC INTERMITTENT ETHANOL IN RATS AFFECTS [3H]RO15-4513 BINDING TO DIAZEPAM-INSENSITIVE GABA-RECEPTORS. D.M. Sapp, S.K. Kokke, and N.W. D'Amico, Dept. of Pharmacology, Brain Research, University of California, Los Angeles, CA 90024.

Chronic intermittent ethanol (CIE) administered to rats produces a 'kindling-like' phenomenon in which the PTZ-induced seizure threshold is decreased. A change in PTZ-induced seizures sensitivity suggests that the GABA receptor complex is involved in the differences observed between control and CIE animals. GABA receptor binding was compared between the two groups of animals using the partial inverse-agonist \(^{[7]}\)HRO15-4513. This ligand was used because of its reported 'alcohol antagonist' activity and its unique binding to both diazepam-sensitive (DZ-S) and diazepam-insensitive (DZ-IS) sites. Crude membrane homogenates were prepared from both the cortex and cerebellum. Several differences in binding were observed between the two groups of animals. In the cerebellum of CIE animals, the binding of RO15-4513 to DZ-S sites is increased while the binding to DZ-IS sites is decreased, i.e., the ratio of DZ-S is decreased. The PET analysis indicates that the affinity of RO15-4513 for the DZ-IS sites is decreased two-fold. The ability of diazepam to displace RO15-4513 binding is also increased in CIE animals. Chronic ethanol thus produces a change in the properties or amount of DZ-IS binding sites for RO15-4513, possibly mediated by a switch in subunit gene expression.

564.7 ALTERATIONS IN TASTE REACTIVITY TO ALCOHOL IN RATS GIVEN CONTINUOUS ALCOHOL ACCESS FOLLOWED BY ABSTINENCE. P.J. Rice and S.W. Kleier, Dept. of Psychology, Kansas State University, Manhattan, KS 66506-3302.

Taste reactivity tests were used to examine the orofacial responses of rats (n=16) to the taste of alcohol during four tests: the first test was before alcohol experience; the second test was done after four weeks of continuous access to 10% alcohol (and water); the third test occurred after two weeks of zero alcohol access; the fourth test was then done four weeks later, during which time the rats received water only. For each reactivity test, rats were presented with 20%, 30%, and 40% alcohol and their orofacial reactivity videotaped and then scored for ingestive and aversive responding. Results showed that the number of ingestive responses recorded during test four, after the alcohol had been removed for four weeks, was almost identical to that of the first exposure. Aversive responses tended to decrease from test one to test three but then increased slightly during test four. It was concluded that the palatability of alcohol increased with continued consumption and then returns to pre-experience levels if rats are without alcohol for a period of time. Supported by NIAAA grant AA07185.

564.8 HEIGHTENED EMOTIONALITY INDUCED BY ETHANOL WITHDRAWAL IS BLOCKED BY INTRA-AMYGDALA INFUSION OF A HELICAL CRF\(\_\)\(_\)41. S.C. Heinrichs, S. Rassnick, K.T. Bruiser, and D.M. Koff. Dept. of Neuropharmacology, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd. and Dep. of Psychiatry, VA Medical Center, La Jolla, CA 92037.

A previous study in our laboratory demonstrated that the Elevated Plus-Maze is a sensitive measure of increased reactivity associated with ethanol withdrawal. Withdrawal of ethanol from chronically intoxicated rats reduced exploration of exposed, lighted walkways in favor of enclosed, darkened arms of the maze. This reduction in time spent on the exposed arms reflects a heightened anxiety-like response produced by ethanol withdrawal and was blocked by low doses of a corticosterone-releasing factor antagonist, a-helical CRF\(\_\)41, administered centrally (Baillie et al, Psychopharmacology 103:277-281, 1991). The present study assessed whether a similar decrease in time spent on the exposed arms occurs at eight hours post-ethanol consumption. The most effective intra-amygdala dose (250 ng ICV) did not affect the behavior of ethanol withdrawn rats when administered intracerebroventricularly (250 ng ICV). Similarly, 250 and 500 ng intra-amygdala doses of CRF\(\_\) antagonist did not affect the Plus-Maze performance of non-dependent rats. These results suggest that a specific, CRF-containing limbic system nucleus may participate in the maladaptive state of behavioral hyperreactivity present during ethanol withdrawal in humans. This research was supported in part by grants DGT 26741 and AA 06420 to O.P.K., AA 02597 to S.R. and the Alcoholic Beverage Medical Research Foundation.

Although alcohol is presumed to be immunosuppressive, there is no agreement regarding its effects on immune responses. The present study determined the effect of chronic alcohol consumption on natural killer cell (NK) activity measured by 51Cr labeled Yac–1 cell lysis and on concanavalin A (Con A) induced lymphocyte proliferation. Male Wistar rats were implanted with gastronomy catheters which permitted continuous intragastric infusion of liquid diet and ethanol for six weeks. The dose of ethanol was 30% of caloric intake which progressively increased to 40% and resulted in mean blood alcohol levels of 240 mg/100 ml. Control rats received isoalcoholic dextrose instead of ethanol. Our result showed that: 1) NK activity was 50 to 60 percent increased and, 2) proliferative response with Con A was two to three fold increased in alcoholic rats. Both alterations were statistically significant at p < 0.05. The increase in Con A response may suggest an increase in IL–2 production following chronic alcohol consumption and may be a contributing factor in the elevation of NK cytotoxicity. (Supported by NIH Grant NS 24008).


Profound and distinct alterations demonstrated that ethanol (ETOH) increases norepinephrine (NE) turnover in the brain. The purpose of this investigation was to assess the effects of the alpha-2 adrenergic receptor antagonist, yohimbine (YOH), on the behavioral manifestations of ETOH intoxication in healthy human subjects. METHODS: 12 subjects participated in 4 double-blind test conditions: placebo ETOH (2.8 ml of 95% ETOH) injected on an appropriate p.o. schedule vs placebo i.v. injection. Placebo ETOH injection, without alcohol, symptoms of anxiety (Panic Attack Symptom Scale (PASS)), and a subjective feeling of euphoria-high were obtained at baseline and throughout the study. RESULTS: The Global Analysis of Variance (ANOVA) showed no significant effects of the ALCOHOL x TIME interactions for total SS score (p>0.001), total PASS score (p>0.001), and for self-ratings of high (p>0.001). The ANOVA at YOH condition vs the ETOH/placebo condition revealed significant YOH x TIME interactions for total SS score (p<0.001), self-ratings of high (p<0.001), and a trend for total PASS score (p<0.17), with the ETOH/YOH conditions. CONCLUSIONS: The combination of YOH-induced increases in NE and ETOH produces a significantly greater degree of intoxication effects of the alpha-2 adrenergic receptor antagonist, yohimbine (YOH). The results suggest that an attenuation of E-I sleep times in LS mice by 30-40%. This BH4 or 6-MPH4 treatment appears to be involved in the regulation of ETOH-induced increases in NE and ETOH produces a significantly greater degree of intoxication.
EFFECT OF ETHANOL AND ATROPINE ON REACTION TIME IN RATS

Male Sprague-Dawley rats were intrastrially injected with either ethanol (EtOH) or atropine (ATR) in order to study the effects on reaction time (RT). RT was measured in msec every 15 min. for 2 hrs. Placement of cannulae was possible, in response to a light and buzzer in order to avoid a mild electric shock. The RT response is mediated in part by the cholinergic function of the striatum, and atropine (0, 0.8, or 8 ug in 1 ul 0.9% saline) was found to slow RT even more. ATR abolished the stimulant response of FAST mice to EtOH, whereas EtOH plus ATR enhanced this interference. It appears that the RT slowing of ethanol has resulted in alterations in response to opiates. The possibility that dopamine (DA) systems mediate ethanol-induced hypothermia may be that neurotransmitter systems other than DA and NE receptors are involved in this response. Alternatively, it may be that neurotensin systems other than DA and NE are involved. 

Supported by NIAAA grants AA07702, AA07469, and AA05828.

GENETIC SIMILARITIES BETWEEN ETHANOL- AND NICOTINE-INDUCED CONDITIONED TASTE AVersion.

Inbred C3H/2lbg and C57BL/6J/lbg) were tested for differences in conditioned taste aversion and that these differences are genetically controlled. Male NIH Swiss mice were pretreated with pertussis tox in (0.5 and 1.0 mg/kg, i.c.v., 7 days prior to testing). These pretreatments reduced the ataxia induced by 2.4 g/kg dose of ethanol. Pertussis toxin treated animals also exhibited a diminished hypothermic response to ethanol (2 g/kg), although the toxin treated animals had lower body temperatures prior to ethanol administration compared to sham treated animals. The binding oligomer of pertussis toxin, which lacks the enzymatically active protomer subunit, was inactive. These results suggest that pertussis toxin sensitive G-proteins may modulate some of the behavioral and physiological effects of ethanol.

Supported by DA-00116 and AA-06391.

EVIDENCE THAT G-PROTEINS MODULATE BEHAVIORAL AND PHYSIOLOGICAL ACTIONS OF ETHANOL.

Mice selectively bred for sensitivity (COLD line) or insensitivity (HOT line) to ethanol-induced hypothermia were used. Evidence was found indicating that common genes control the aversive properties of these two agents. Recently, a number of neurotransmitter receptors (for example, DA, adenosine A1, GABA, and others) linked to their effector mechanisms by pertussis toxin sensitive guanine-nucleotide binding proteins (G-proteins) have been postulated to modulate ethanol-induced conditioned taste aversion and that these differences are dose dependent. Furthermore, similarities exist among the inbred strains for conditioned taste aversion whether nicotine or ethanol is the unconditional stimulus. This finding may indicate that common genes control the aversive properties of these two agents. 

Supported by DA-00116 and AA-06391.

EVIDENCE OF THE ETHANOL-INDUCED TASTE AVERSION IN Mice SELECTED FOR DIFFERENTIAL SENSITIVITY TO ETHANOL.

Mice selectively bred for sensitivity (COLD line) or insensitivity (HOT line) to ethanol-induced hypothermia were characterized using taste conditioning. Fluid deprived COLD and HOT mice were given six taste training trials at 48-h intervals. On each trial, access to a saccharin solution was followed by injection of levorphanol (3 mg/kg on trials 1-4, 6 mg/kg on trials 5-6). Saccharin-levorphanol pairing produced aversion that was greater in HOT mice. Rectal temperatures determined before injection and 60 min after injection revealed greater ethanol-induced hypothermia in COLD mice. This data support previous findings suggesting that genetic selection for sensitivity/insensitivity to ethanol has resulted in alterations in response to opiates. Also, this outcome is consistent with the notion that drug-induced hypothermia is related to drug's hedonic effects.

Supported by NIAAA grants AA07649 and AA08621.

EVIDENCE THAT G-PROTEINS MODULATE BEHAVIORAL AND PHYSIOLOGICAL ACTIONS OF ETHANOL.

Mice selectively bred for sensitivity (COLD line) or insensitivity (HOT line) to ethanol-induced hypothermia were characterized using taste conditioning. Fluid deprived COLD and HOT mice were given six taste training trials at 48-h intervals. On each trial, access to a saccharin solution was followed by injection of levorphanol (3 mg/kg on trials 1-4, 6 mg/kg on trials 5-6). Saccharin-levorphanol pairing produced aversion that was greater in HOT mice. Rectal temperatures determined before injection and 60 min after injection revealed greater ethanol-induced hypothermia in COLD mice. This data support previous findings suggesting that genetic selection for sensitivity/insensitivity to ethanol has resulted in alterations in response to opiates. Also, this outcome is consistent with the notion that drug-induced hypothermia is related to drug's hedonic effects.

Supported by NIAAA grants AA07649 and AA08621.
The decrease in ethanol self-administration resulting from when compared to the effects of DA agonists females showed increased DA levels in both nucleus accumbens (peaking of DA, DOPAC and HVA in nucleus accumbens and striatum in adult male accumbens (e.g., DiChiara & Imperato, 1988). Using brain microdialysis, we 

drugs of abuse, are related to dopamine (DA) release in the nucleus 

neurochemical response to ethanol and its reinforcing 

when compared to the effects observed with systemic administration 

of water (30 min daily sessions). Bicuculline methylchioride, a competitive GABA antagonist, selectively reduced E reinforced responding, (2.5, 5, 10, & 20 ng; 5 ng as the most effective dose), without altering water responses. A NMDA-selective glastine antagonist, 2-amino-5-phosphonovaleric acid (APV) also suppressed E responding (1.5, 3, & 6 μg; 1.5 μg as the most effective dose). Doses of 3 and 6 μg produced a trend toward suppressing responding for both E and water. These results contribute further support for GABA and glutamate as neurochemical mediators of the acute reinforcing effects of E and suggests that the underlying neural circuitry includes the amygdala as an anatomical substrate (Supported in part by AA06263 & RR08016).

The concurrent abuse of ethanol (E) and cocaine has been recently been frequently reported clinically. This combination has been shown to produce cocaethylene (CE), the ethyl ester of benzylecgonine. This compound has been found to be self-administered by rats in doses that potentiate and to also block reuptake and increase the release of dopamine in rats. The present study examined the effects on E SA of CE on drinking and drinking rats. Animals learned to consume increasing concentration of E over several weeks using a limited access procedure. All rats showed a preference for 11% E over water. CE (1.0 mg/kg, ip) injection increased E and water consumption. A higher dose (2.5 mg/kg, ip) of CE decreased E consumption while not affecting water intake. The effects of CE on E SA appear similar to those of psychomotor stimulants.

(Supported in part by AA06263 & RR08016)

565.9 EFFECT OF SEROTONIN ANTAGONISTS ON THE DEXENFLURAMINE (D)-INDUCED ATTENUATION OF ETHANOL INTAKE IN WISTAR RATS. D.M. Tonning*, E.M. Sellers and G.A. Higgins. Deps. of Pharmacology & Medicine, University of Toronto and Clinical Research & Treatment Institute, Addiction Research Foundation, Toronto, Ontario, Canada M5S 2S1.

Using a computerized drinkometer system, we recently demonstrated that D, a 5-HT receptor antagonist, reduces ethanol intake in a dose-related manner in rats allowed continual access to water and 5% ethanol (Hoggins et al. JPET, submitted). The aim of the present study was to assess which 5-HT receptor subtypes may be involved in the mediation of this effect. Ethanol-prefering rats receiving saline injections consumed between 2.4-3.3 g/kg ethanol during the 12 hr dark phase. D (1 mg/kg s.c.) 1 hr prior to injection produced a marked attenuation (29%, p < 0.01) in relation to food (4%, p < 0.01) intake. The reduction in ethanol intake was due to an increase in the latency (p < 0.01) and a reduction in the number of drinks (p < 0.01), and 50% were unaffected. The reduced ethanol intake could be reversed by pre-treatment with either metergoline (1 and 5 mg/kg) or ritanserin (1 and 3 mg/kg, p < 0.01) which when given alone had no significant effect on ethanol intake. Ondansetron (0.01, 0.1 and 1 mg/kg) and xylamidine (3 mg/kg) had no significant effect on D-induced reduction in ethanol intake. However, 0.1 mg/kg ondansetron and xylamidine (2 mg/kg) had no effect on ethanol intake by 17% (p < 0.05). The reversal of the D-induced reduction of ethanol intake by the non-selective 5-HT antagonists metergoline and the 5-HT TC2 antagonist ritanserin, but not by either ondansetron (5-HT1 antagonist) or xylamidine (peripheral 5-HT antagonist) suggest a role for central 5-HTTC2 receptors in the mediation of this effect.

565.11 ANGIOTENSIN II AND CAPTOPRIL ENHANCE ETHANOL INTAKE IN RATS. D.A. Flits. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Chronic infusions of angiotensin II into the lateral ventricles for water intake at 5 and 20 pmol/hr and increased intake of 6% (v/v) ethanol at 20 pmol/hr relative to baseline values and to vehicle-infused controls. Captopril placed into the drinking fluid at 50 mg/kg for 4 days significantly increased intake of 4 to 6% ethanol in three of four experiments and never significantly reduced ethanol intake. Functionally identified lesions of the subfornical organ (SFO) totally abolished captopril-enhanced water intake but did not appear to affect the increased intake of 6% ethanol during captopril treatment. The data appear to reflect a general increase in fluid intake rather than a specific appetite for alcohol, or alcohol taste, such as would be expected with NaCl solutions, but this increase with captopril appears to be independent of the SFO. Our results with angiotensin and captopril were opposite to previously published reports by others, and the data question whether it is appropriate to use converting enzyme inhibitors to treat problem drinking in humans (e.g., Spinosa, G., et al., Alc. Clin. Exp. Res., 12, 65-70, 1988). Supported by NS-22274.


We have found that ethanol (E) produced dose-related reductions of wheel-running (WR) performance. RO, an inverse agonist has been found to antagonize the E-induced suppression of several behaviors. Recently, we have found that RO (0.063-3.00 mg/kg, ip) failed to antagonize the depressant actions of experimenter-administered (EA) E (0.75 g/kg, ip) on WR.

The present study examined the effects of orally self-administered E on WR and of RO (2.5 & 5.0 mg/kg) effects on these behavioral responses. Rats who had acquired a preference for E were given 10 min tests of WR following intake of E (11% v/v) during a two-bottle choice of E and water. Unlike with EA injection, oral intake of 1.6 g/kg of E did not suppress WR. RO alone and in combination with oral E did substantially reduce WR similar to EA E. These data suggest that route of administration plays an important role in the effects of E on WR. Moreover, RO suppresses WR behavior more strongly in animals with E independent of route of administration.

(Supported in part by AA06263 & RR08016)


Stress is accompanied by a rise in the secretion of adrenocortical hormones and it may be considered whether or not these hormones, and especially corticosterone, exert an influence on ethanol consumption. In the present investigation this issue was addressed by studying the effect of adrenalectomy (ADX) and subsequent corticosterone treatment on ethanol intake. During a three week period before ADX the intake of water and 6% (w/v) ethanol was recorded for 60 male rats. Based on their individual preferences for ethanol, the rats were partitioned into mid-range, low and high ethanol (HE) and one sham-operated group. ADX rats received a 0.9% saline solution instead of water and 6% ethanol solution based upon a 0.9% saline solution. Fluid intake was recorded for 8 days after ADX and sham-operation. The ADX and control groups were each divided in two subgroups. The subgroup from ADX rats and one from the sham-operated rats received corticosterone in their fluid bottles (25 mg/1000 ml fluid) according to a method developed by Arora, S. F. et al. (Am. J. Physiol. 1985, 249, R527-R532). The remaining two subgroups served as controls for the corticosterone treatment. Fluid intake was recorded for another period of 8 days. The results showed that ADX rats significantly decreased their ethanol intake and preference compared to the sham-operated controls. There was no difference between groups in total fluid intake. Treatment of ADX rats with corticosterone caused a significant increase of ethanol intake and preference compared to the ADX controls. Corticosterone restored the intake of ethanol to a normal level. This suggest that corticosterone is involved in the regulation of ethanol consumption.


Cholecystokinin octapeptide (CCK-8) is a brain-gut neuropeptide shown to inhibit intake of food and alcohol in a variety of species. Recently, Draski and colleagues developed strains of rats differing in sleep and wake behavior after acute injection of ethanol. We measured the intake of 5% alcohol, food and water in water-deprived rats with high, low, and control alcohol sensitivity (HAS, LAS, and CAS), after ip injection of CCK-8 (0-8 μg/kg). LAS rats consumed significantly less alcohol than HAS or CAS rats, which did not differ reliably. Injection of CCK-8 significantly and equivalently inhibited alcohol intake in each group of rats. Water intake following alcohol was unaffected by prior CCK-8 injection. Differences in alcohol sensitivity in these rats cannot be attributed to differential sensitivities to CCK-8. However, differences in alcohol sensitivity may be inversely related to sensitivity to alcohol's anesthetic effect. (Supported by NIH Grant No. RR-08197).
565.13


Several lines of evidence suggest that the CNS serotonin (5HT) systems have been observed to alter alcohol consumption. Compared with rats of the alcohol-nonpreferring NP line, P rats have been shown to have lower levels of 5HT in most forebrain regions and an upregulation of some 5HT receptor subtypes. The present study tested the effects of oral administration of subchronic injections of buspirone, a 5HT1A agonist (1.0-9.0 mg/kg), and two 5HT antagonists, MDL12222 and ICS205930 (0.01-3.0 mg/kg). Adult female rats (n=8/group) were given ad lib access to food and water, but access to a 10% (v/v) ethanol solution was restricted to 4 h/day. Rats were first habituated to saline injections. Each drug dose was given before alcohol access on 4 consecutive days. Rats were given saline injections and returned to baseline intakes on days between drug doses. Buspirone caused a dose-dependent decrease in alcohol intake, mostly during the first hour of ethanol access (p<0.001 compared with saline). MDL12222 and ICS205930 did not significantly alter ethanol intake. The findings indicate a role for 5HT1A receptors in alcohol drinking of P rats, but no evidence for the involvement of 5HT1B receptors was obtained. (Supported by AA07611 and AA08553)

565.15


Studies in men at genetically increased risk for the development of alcoholism have shown that they may have a qualitatively different response to acute doses of ethanol. Differential sensitivity to the acute effects of ethanol has also been found in alcohol-preferring (P) and non-preferring (NP) rats. To generalize these neurophysiological findings to the human subject, we investigated the acute effects of ethanol as well as diazepam and the noncompetitive NMDA receptor antagonist MK-801, on the acute and chronic consumption of ethanol. EEG activity was measured in P and NP rats using EEG and auditory event related potentials (ERP) recordings. P and NP rats were implanted with electrodes in cortex (CTX), dorsal hippocampus (DHPC), thalamus (THAL), and amygdala (AMYG). Rats were trained in discrimination tasks (AMYG, CTX, and DHPC) following vehicle administration, in that P rats had more power in the low (1-2 Hz) and high (32-64 Hz) frequency ranges, reduced theta frequency (6-8 Hz) in the EEG, and reduced N1 component amplitudes in CTX and DHPC. In response to ethanol, P rats differed from those in NPs in that they displayed less EEG slowing and less reduction or increases in ERP component amplitudes. No EEG or ERP differences were found in response to diazepam in P and NP rats. In response to MK-801, P rats did demonstrate lower wave and slow waves or reductions or ERP component amplitudes. These studies indicate that in addition to differences in baseline, P rats have reduced electrophysiological effects of both ethanol and MK-801, suggesting that differential response of brain glutaminergic systems may underlie differences in acute sensitivity between P and NP rats. (Supported by AA00909, 06059, 07611)

565.16


EEG electrophysiological measures have been shown to differ in human subjects at genetic risk for alcoholism both prior to and after challenge doses of ethanol. In the present study EEG was recorded from indwelling electrodes in rats which were selectively bred for alcohol-preferring (P) and non-prefering (NP) behaviors during an ethanol self-administration paradigm. Initially, 0.2% saccharin solution was available on a FR1. Ethanol was then added to the saccharin solution in increasing concentrations which was found to be an effective conditioned reinforcer. Ethanol EEG recordings were obtained in three conditions: 1) 0.2% saccharin 2) 5% ethanol and 3) 10% ethanol. Differences in mean power and peak frequency were statistically compared. P rats were found to have overall increases in EEG special power in the theta frequency ranges in all experimental conditions (4-6 Hz, p<0.006; 6-8 Hz, p<0.007). Ethanol self administration, as compared to saccharin, was found to produce an increase in mean EEG power in both P and NP rats in all frequency bands (4-6 Hz, p<0.006; 6-8 Hz, p<0.002; 8-16 Hz, p<0.01; 16-32 Hz, p<0.001), and a decrease in peak frequency in 3 of the bands (4-6 Hz, p<0.006; 6-8 Hz, p<0.002, 8-16 Hz, p<0.04). The pattern of EEG changes observed following ethanol consumption differed in the P vs. NP rats, and paralleled their consumption patterns. In NP rats, EEG changes were more prominent in the 5% condition and decreased in the 10% condition where they pressed little for alcohol. In contrast, P rats demonstrated the largest EEG effects in the 10% condition concomitant with increases in their consumption rates. These studies suggest that P rats may need to drink more alcohol to experience the same EEG effects as NP rats. This finding may represent part of the substrate for the difference in alcohol preference between the two rat lines. (Supported by AA00699)

565.17


Motor activity is increased following low to moderate doses of ethanol, but not in NP rats. The present study tested P and NP rats for differential sensitivities of EEG changes observed following ethanol consumption however differed in the P vs. NP rats, and paralleled their consumption patterns. In NP rats, EEG changes were more prominent in the 5% condition and decreased in the 10% condition where they pressed little for alcohol. In contrast, P rats demonstrated the largest EEG effects in the 10% condition concomitant with increases in their consumption rates. These studies suggest that P rats may need to drink more alcohol to experience the same EEG effects as NP rats. This finding may represent part of the substrate for the difference in alcohol preference between the two rat lines. (Supported by AA00699)

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The densities of 5-HT recognition sites labelled with [3H] LY 278584 were determined in the CNS of alcohol-naive, adult male rats (N=4 each) from the alcohol-preferring (P) and non-preferring (NP) lines by quantitative autoradiography. Coronal sections (20 um), prepared from frozen brains, were incubated with [3H] LY 278584 for 1 hour at room temperature in a Tris-HCl buffer containing 2.0 nM [3H] LY 278584. Among the cortical areas, the highest densities (16.17 fmol/mg prot) were observed in (a) layers 1 and 2 of the medial prefrontal and cingulate, (b) piriform and (c) entotinical regions. In the nucelus accumbens and ventral hippocampus, low binding (4-9 fmol/mg prot) was observed in the nuclei and were not different in the densities of 5-HT, sites between P and NP rats. The results do not indicate any abnormality in CNS 5-HT, receptors which could be associated with high alcohol drinking behavior. (AA08553, AA07462, AA07613)
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565.19


Ro15-4513 (RO), an inverse BDZ agonist, has been shown to block ethanol administration in selectively bred ethanol (E) preferring P and non-preferring NP rats. This compound has also been found to antagonize ethanol locomotor effects in unselected rats. The present study investigated the effects of RO on locomotor activity in P and NP rats. P rats initially showed higher levels of activity than NP rats during baseline measurement of activity. Projections (24 h) of RO (2.5 and 5.0 mg/kg) and E (0.75 g/kg) each were given alone and in combination with each other prior to testing. E alone reduced activity in both P and NP rats, although P rats showed less reduction. RO alone and in combination with E produced greater suppression of locomotor activity in both rats than E alone. These data show that RO does not antagonize E-induced locomotor depression in P and NP rats. Moreover, they demonstrate the intrinsic decrease in the effect of E and the lack of generality of its effects in blocking E's action.

(Supported in part by AA06263 and RR08016)

565.20

565.21


Offsetting ethanol hypothermia influences behavioral sensitivity to ethanol. The direction of the response depends upon genotype, with ethanol sensitivity increasing in C57 and decreasing in LS mice. Genetic differences also exist in the effects of ethanol on GABA-stimulated chloride (Cl) channel activity. These differences are consistent with selectively bred differences in behavioral sensitivity to ethanol. Therefore, we hypothesized that changes in GABA receptor activity induced by ethanol offsetting hypothermia might be due to an interaction of temperature and ethanol at the GABA, nicotinic receptor complex (GBRC). We found that increasing in vitro temperature from 30 to 38°C eliminated the significant effect of ethanol (25-100 mM) on GABA-stimulated Cl uptake in LS mice, which was present at 30°C. In contrast, there was no significant effect of ethanol (50-200 mM) or temperature (30 & 38°C) on GABA-stimulated Cl uptake in C57 mice. These results suggest that an interaction between temperature and ethanol at the GBRC might explain the negative relationship between temperature and ethanol sensitivity found in LS mice, but it cannot explain the positive relationship between temperature and ethanol sensitivity found in C57 mice. (Supported by U.S.P.H.S. grants AA03972, AA05234, NS25986 & NS24645.)

565.22

PHARMACOLOGICAL AND NEUROCHEMICAL STUDIES IN HOT AND COLD MICE. J.F. Stuart*, J. Dorow*, J.C. Crabbe and D.J. Feller. VA Medical Center and Oregon Health Sciences University, Portland, OR 97201.

Mouse lines are being selected which are sensitive (COLD,2) and resistant (HOT,1,2) to ethanol induced hypothermia. HOT/COLD mice (both replicates) were tested for hypothermic sensitivity to drugs acting through receptor or ion channel specific mechanisms: dexmedetomidine, a2-adrenergic agonist; adenosine agonist; caffeine, adenosine antagonist and diuretics; calcium channel blocker. COLD mice (both replicate lines) were significantly more sensitive than HOT mice to diltiazem, but not dexmedetomidine. C2 mice were more sensitive to adenosine drugs than H2 mice, while H1/C1 mice were equally sensitive. The diltiazem data suggest that calcium channel function may be involved in ethanol's effects on body temperature. We previously showed that opiate agonists are more potent in COLD compared to HOT mice. Receptor binding with [3H]DAMGO, a μ-opiate agonist, and morphine inhibition of forskolin stimulated adenylyl cyclase were studied in frontal cortex of COLD compared to HOT mice. Receptor binding with [3H]DAMGO, a μ-opiate agonist, and morphine inhibition of forskolin stimulated adenylyl cyclase were studied in frontal cortex of COLD compared to HOT mice. Morphine inhibition of adenylyl cyclase was similar in both mouse lines for the frontal cortex and other brain areas. These data suggest that differences between HOT/COLD mice in sensitivity to opiates drugs is not mediated through changes in μ-opiate receptor function.

These studies were supported by Grants AA05828, NIDA Contract 271-87120, AA01548 and AA08621.

566.1


The discriminative-stimulus (DS) effects of cocaine alone and after pretreatment with selected opioids were determined in squirrel monkeys using a two-lever choice procedure. When tested alone, cocaine engendered dose-related increases in cocaine-appropriate responding, reaching a maximum of 97-100% at doses of 0.5 mg/kg or greater. Pretreatment with the μ agonists morphine, levorphanol, and methadone and the partial agonist buprenorphine, partly attenuated the DS effects of cocaine such that the cocaine dose-effect curve was shifted to the left by as much as 30-fold. In contrast pretreatment with the κ agonist MR2266 (0.03-0.5 mg/kg) partially attenuated the DS effects of cocaine, shifting the cocaine dose-effect curve to the right by up to 3-fold. None of the opioid agonists antagonized the κ agonist naltrexone. Pretreatment with the opioid antagonists naltrexone and MR2266 did not alter the DS effects of cocaine. Naltrexone did, however, antagonize the cocaine-potentiating effects of both morphine and buprenorphine. The results show that activation of μ and κ receptors can modulate the reinforcing effects of cocaine in different ways, which may reflect their opposing influences on dopamine release. Supported by DA00538, DA00496, DA03747, MR03756 and RR01886.

566.2


Recent reports indicate that buprenorphine, an opioid agonist/antagonist, suppresses self-administration of cocaine in rats and enhances cocaine's reinforcing effects in an taste preference procedure in rats. Given these findings, the present study was conducted to evaluate the effects of buprenorphine on another component of cocaine's action, namely its discriminative stimulus effects. Using a two-lever drug discrimination procedure, rats were trained to discriminate a dose of 1.0 mg/kg cocaine from a low dose of dopamine. After substitution tests, both cocaine (1.0-10.0 mg/kg) and d-amphetamine (0.1-3.0 mg/kg) substituted for the cocaine stimulus in a dose-dependent manner. In contrast, buprenorphine (0.03-0.5 mg/kg) and naltrexone (1.0-10.0 mg/kg) failed to substitute for the cocaine stimulus, up to doses that substantially decreased rate of responding. Across the dose range examined, buprenorphine (0.03-0.5 mg/kg) failed to alter the stimulus effects produced by cocaine or d-amphetamine. Morphine (0.3-10.0 mg/kg) and naltrexone (1.0-10.0 mg/kg) also failed to alter cocaine's stimulus effects. Thus, although buprenorphine may alter the reinforcing effects of cocaine, the present study demonstrates that buprenorphine does not alter the stimulus properties of cocaine in rats. (Supported by P.H.S. grants DA 07244 and DA 02749).

DRUGS OF ABUSE—COCAINE: PHARMACOLOGY

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1425

BRAIN AND PITUITARY β-ENDORPHIN SYSTEM OF THE AA AND ANA RATS. J.P. De Wilde, E. Kilgamar* and C. Giampulakis. Douglas Hospital Research Centre, McGill University, Verdun, Québec; Research Laboratories, Alko ltd. Helsinki, Finland.

Genetically determined differences in the activity of specific components of the endogenous opioid system may be partially responsible for the preference or aversion to ethanol exhibited by selectively bred ethanol preferring (AA) and non-ethanol preferring (ANA) rats. In the present study the content of PM β-endorphin in the pituitary and hypothalamus as well as of immunoreactive β-endorphin (ir-β-EP) in distinct regions of the hypothalamus and the pituitary gland of the alcohol-prefering AA and alcohol avoiding ANA rats were measured under basal conditions. The content of PM β-endorphin was significantly higher in the septum and significantly lower in the amygdala and periaqueductal grey matter of the AA rats, while there was no significant difference among AA and ANA rats in the arcuate nucleus, nucleus accumbens, caudate, hippocampus and cortex. The content of PM β-endorphin but not of ir-β-EP was significantly higher in the hypothalamus and neuro-intermediate lobe of the AA rats, while in the anterior pituitary the content of both ir-β-EP and PM β-endorphin were significantly higher in the AA than ANA rats. Thus there are specific differences in the brain and pituitary β-endorphin systems among the AA and ANA rats. The importance of these differences on controlling the voluntary ethanol consumption is under investigation.

566.1

566.2
REINFORCED BY INTRAVENOUS COCAINE IN THE RAT

566.7

There is substantial evidence to support the hypothesis that dopamine receptor stimulation is essential for psychomotor stimulant effects in the typical neuroleptic, clozapine, is a dopamine antagonist, one would predict it should attenuate the reinforcing effects of cocaine. This was tested by evaluating the effects of clozapine (5-20 mg/kg) on Wistar rats trained to self-administer cocaine (0.6 mg/linj) on a Progressive Ratio schedule of reinforcement.

Paradoxically, clozapine was found to substantially increase breaking points indicating that it in fact potentiated cocaine's reinforcing effects.

Clozapine has actions on other transmitter systems (i.e. serotonin) which are likely responsible for this atypical response.

(Supported by NIDA grants DA-00490 and DA-07027 and the Commonwealth of Virginia Center for Drug Abuse Research)

566.8

Interactions have been reported between cocaine and stress. Specifically, stress has been shown to sensitize animals to the motor activating effects of cocaine. Cocaine and stress are proposed to interact with monaminergic systems. If the behavioral sensitization of cocaine by stress is due to similar mechanisms then cocaine and stress may potentiate the behavioral effects of stress. The goal of the present experiment was to determine the effects of chronic cocaine administration on the behavioral effects of chronic stress.

Male C57B1 mice were placed on activity monitors for 1 hour for 14 days. One-half of the animals were stressed using an acoustic stress consisting of 1 sec of a 105 dB, 8 kHz tone presented on a variable time 90 sec schedule (0-80 sec range). These hours following the session half of the sessions were on a 25 mg/kg cocaine HCl or saline. There were four treatment groups: CON - no stress, vehicle. COC - no stress, cocaine. STRESS - stress, vehicle. STRESS-COC - stress, cocaine.

CON animals showed a similar level of activity throughout the 14 days of study. STRESSED animals showed an enhanced locomotor activity which varied between 125% (Day 10) and 230% (Day 13) of control. COC animals, administered 3 hrs after the session half of the sessions were on a 25 mg/kg cocaine HCl or saline. These data do not support a sensitization of the behavioral effects of stress. The goal of the present experiment was to determine the effects of chronic cocaine administration on the behavioral effects of chronic stress. Male C57B1 mice were placed on activity monitors for 1 hour for 14 days.

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566.6

The mixed opioid agonist-antagonist buprenorphine (BUP) can reduce cocaine self-administration in monkeys. Studies were done to help clarify the manner in which buprenorphine can reduce cocaine self-administration behavior. Studies in progress are investigating the ability of BUP to attenuate drug discrimination in rats. Results indicate that a dose of 100 μg/kg (administered i.p. 30 min before testing) significantly attenuates cocaine self-administration. The effects of lower doses are under study. Additional experiments have assessed the effects of BUP on both cocaine and morphine drug-discrimination in rats trained to discriminate drug from saline.

BUP (0, 1, 10, 100 μg/kg) was effective in increasing the percentage of rats selecting the drug lever from days 5-14. These data do not support a sensitization of the behavioral effects of stress.

566.5

In previous studies, local anesthetics have been shown to have cocaine-like discriminative stimulus effects. For the present study a two-lever drug discrimination procedure was used to train rats (N=6) to discriminate between 10 mg/kg cocaine (s.c.associated with one lever) and either 55 mg/kg procaine or saline (associated with the other lever) in order to separate the local anesthetic effects of cocaine from its central stimulatory effects. After the subjects were trained, each situation was conducted with either cocaine (1.0-30 mg/kg), procaine (0.1-110 mg/kg), lidocaine (1.0-30 mg/kg), or mepipvacaine (0.1-3.0 mg/kg). Cocaine, lidocaine, and mepipvacaine generalized comparable effects of the training dose of cocaine in a dose-dependent manner. Procaine showed no generalization from cocaine, and lidocaine showed little cocaine-lever responding, even at doses that disrupted behavior. These results provide further evidence that cocaine discrimination is not based on its local anesthetic effects and that lidocaine, which has high affinity for the cocaine recognition site in the dopamine transporter, is not more similar to cocaine than procaine.

(Supported by NIDA grants DA-00490 and DA-07027 and the Commonwealth of Virginia Center for Drug Abuse Research)

566.4
EFFECTS OF ALPRAZOLAM ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN RATS. N.E. Goeders, M.A. McNulty and G.F. Guerin. Dept. of Pharmacology & Therapeutics, LSU Medical Center, Shreveport, LA 71130-3932.

The following experiments were designed to investigate the effectiveness and specificity of alprazolam on intravenous cocaine administration. Alprazolam was investigated since this drug has been known to be clinically effective in the treatment of anxiety and panic attacks and has been proposed to be useful in the treatment of some types of depression. Alprazolam has been associated with anxiety, depression and even panic attacks in some cases.

Alprazolam was tested under a multiple schedule of intravenous cocaine presentation and food reinforcement. Cocaine (0.5 mg/kg) was available during the first hour of the session under a fixed-ratio 4 schedule of reinforcement. During the second hour, food presentation (45 mg pellets) was available under a discrete-trial fixed-ratio 4 schedule of reinforcement. The animals were pretreated with alprazolam (0.05, 0.5, 1.0, 2.0 and 4.0 mg/kg), i.p. thirty minutes prior to the start of the behavioral session. Initial exposure to alprazolam resulted in no specific decreases in both cocaine- and food-maintained responding. However, although the animals quickly became tolerant to the effects of the drug on food reinforced responding upon subsequent testing with alprazolam, dose-related decreases in cocaine self-administration were maintained. These data suggest that pharmacological properties inherent to alprazolam (i.e. anxiolytic) specifically alter cocaine reinforcement without affecting responding maintained by food. Experiments are currently in progress to determine the nature of these effects by completing dose-response curve with alprazolam and by determining whether the benzodiazepine receptor antagonist, flumazenil (Ro 15-1788)
566.10 THE PROGRESSIVE RATIO PARADIGM AS A MEASURE OF THE REINFORCEMENT STRENGTH OF COCAINE: AN ANALYSIS OF LONG TERM STABILITY OF BREAKPOINT AND DISCRIMINANT RESPONSE RELA-
TIONS. S. Schenk and B. Palter. Texas A&M Univ., Dept. Psych., College Station, TX, 77843.

The progressive ratio (PR) paradigm has been applied to the study of the reinforcing effects of intravenously self-administered drugs. Within a test session, the procedure produces an increasing ratio requirement to obtain drug infusions and a "breakpoint", which is the last ratio that is completed by the animal. If the assumptions of the paradigm are correct and if long-term stability of breakpoints can be achieved, the procedure offers several advantages in the interpretation of the effects of manipulations on the reinforcement impact of drugs of abuse. We examined the stability of breakpoints for cocaine self-administration over an extended period of several weeks. We also examined dose/response relationships for breakpoints at various cocaine doses in the self-administration procedure (0.25, 0.5 and 1.0 mg/kg/inj). The results of 70-80 days of testing revealed that breakpoints failed to reach an acceptable level of stability in that they were inconsistent from day to day. In addition, we failed to observe dose dependence either in terms of breakpoints or in terms of number of reinforcers earned. In contrast, when an FR 1 schedule of reinforcement was introduced, responding was dose/designed and reliable. The data argue that the PR procedure, as used under the present circumstances, lacks the internal validity to qualify as an effective measure of the reinforcing impact of cocaine in an intravenous self-administration paradigm.

566.11 EFFECTS OF MAGNESIUM ON RESPONDING MAINTAINED BY COCAINE AND OTHER RESPONSE MAINTAINING STIMULI: SPECIFICITY AND SELECTIVITY OF CHANGES. K.M.Kantak and T.O.Verberne, Lab. of Behav. Neuroscience, Dept. Psychol., Boston University, Boston, MA 02215

In a variety of behavioral experiments, magnesium has been shown to interact with cocaine and other drugs of abuse. Of particular relevance to the present experiments is the recent finding that magnesium maintains responding in cocaine-trained rats. It would be expected, therefore, that injections of magnesium would have rate-altering effects on self-administered cocaine. Five experiments examined the specificity and selectivity of this relationship. Acute and cumulative injections of magnesium (0.25-250 mg/kg s.c.) produced dose-dependent reductions in responding maintained by cocaine (0.1-2 mg/kg/inj). A magnesium-deficient diet produced a dose-dependent increase in cocaine-maintained responding. Magnesium injections were specific because food-maintained responding was not affected, however, these effects were not selective because glucose + saccharin-maintained responding was affected in a manner similar to cocaine-maintained responding as was responding maintained by extinction from cocaine. These data indicate that magnesium may be either decreasing responding by increasing the reinforcing properties of cocaine and other highly rewarding substances or by suppressing responding by serving as a punishing, aversive or negative stimulus. Supported by DA ROI-4137 and Texas Advance Technology Research #3711.


Triamidine (TDF), a triazole fungicide, has been found to produce acute stimulant-like effects on schedule-controlled behavior and to substitute for cocaine. TDF tolerance to stimulant effects on schedule-controlled behavior frequently develops upon repeated exposures, especially when the initial effect is to decrease reinforcement density. The present study was conducted to determine whether behavioral tolerance would develop when TDF was given repeatedly. Effects of TDF on performance maintained by a multiple fixed-interval 10 fixed-interval 3-minute (multi FR 10 FI 3 min) schedule were determined in twelve male Long Evans rats. Rats were divided into two groups which received 30 mg/kg TDF daily for 15 days and then 56 mg/kg TDF daily for the following 15 days. The remaining four rats received vehicle (VEH) daily for 30 days. Effects of 56 mg/kg TDF on response rates, reinforcement rates, and FI index of curvature (IOC) values were compared before and after daily TDF administration. Acute administration of 56 mg/kg TDF produced decreases in FR response rates, while daily TDF administration differed considerably between rats. These results indicate that tolerance can develop to some of the behavioral effects of TDF.

The potential reinforcing effects of cocaine can readily become associated with salient environmental stimuli, which then acquire secondary reinforcing properties. It has been proposed that the reinforcing properties of these conditioned stimuli (CSs) are due to the ability of the CSs to elicit similar neural events as the drug itself. Given the large body of evidence that implicates the mesolimbic dopaminergic pathway in the rewarding properties of cocaine, the present study was designed to determine whether stimuli paired with cocaine elicit similar neurochemical changes as cocaine, as assessed by in vivo brain microdialysis.

The administration of cocaine (10 mg/kg for 7 days) in association with a specific environment (CS+) produced significant conditioned locomotion in that environment. Rats that received cocaine in their home cage (CS-) did not exhibit increased locomotion on the test day, as compared to subjects that received saline in both settings.

When administered acutely, cocaine (10 mg/kg) produced a potent unconditioned effect on intravisual dopamine (80% of basal values) in the nucleus accumbens. In contrast to the ability of repeated pairing of cocaine with a specific environment to produce conditioned locomotion, there was a lack of evidence for coacquisition of conditioned neurochemical events. Specifically, the modest increase in dopamine (10%) above basal values) observed after exposure to the conditional environmental stimuli was equal in the CS+ and CS- groups. These data do not support the hypothesis that stimuli paired with cocaine produce their behavioral effects by eliciting similar neurochemical effects as cocaine.

566.17 PRAZOSIN AND ONDANSETRON ANTAGONIZE COCAINE'S EFFECTS ON FIXED-INTERVAL AND FIXED-RATIO BEHAVIOR. F. van Hamen. Dept. of Psychology, University of Florida, Gainesville, FL 32611.

Three White Carneaux pigeons pecked a key on a multiple fixed-interval (FI) 2 min, fixed-ratio (FR) 2 schedule of reinforcement. The rats were first habituated to cocaine administration 5 min prior to the start of a session (0.1, 1.0, 1.7, 3.0, 5.0, and 10.0 mg/kg) systemically administered and reinstated FR rates and FI rates in two of the three subjects. FR rates were more affected by lower doses. Response rates in the third subject were not consistently affected by cocaine. Prazosin (alpha1 antagonist) and, later, ondansetron (GR38032F, 5-HT3 antagonist) were co-administered with doses of cocaine which previously decreased response rates. Both prazosin (1.0 mg/kg) and ondansetron (0.10 and 0.50 mg/kg) antagonized cocaine's rate decreasing effects on the FR and FI schedules and reinstated responding where it had previously been totally suppressed. Prazosin and ondansetron in combination with cocaine decreased FR and FI response rates in the subject whose behavior had not been affected by cocaine alone.

566.19 EFFECTS OF CHRONIC COCAINE ON FOOD COMPETITION IN RAT TRIADS. E. El-Awady* AND M.C. Wilson. Dept. of Pharmacol., Sch. of Pharm., Univ. of Mississippi, University, MS 38677.

The purpose of the study was to investigate the effects of cocaine(C) on the competitive behavior of rat triads. Within these triads, rats were ranked during the 4th week of housing according to average competition scores for sucrose pellets. Middle ranking rats were treated daily for 2 wk with C (5.0 mg/kg, IP, b.i.d.). Competition was scored 3-5 hr or 24 hr after drug administration. C increased competition scores mainly in the early testing period resulting in some rats achieving the dominant status in the triad. After treatment, 4 days were designated as a withdrawal period, and 3 additional days as a washout period. The middle ranking rats, based on average competition scores during the washout period, were treated with C (20 mg/kg, IP, b.i.d for 2 wk). This dose failed to affect the competition; however, competition scores of treated animals increased in all triads during the withdrawal period. This increase declined during washout. Therefore, chronic C affected food competition in time- and dose-dependent manners. (Supported by Res. Inst. of Pharm. Sci.)

566.16 THE RELATIVE CONTRIBUTION OF PERIPHERAL AND CENTRAL COMPONENTS IN COCAINE-INDUCED PLACE CONDITIONING. J.E. Hendry, S.H. Jones, D.B. Neil, & J.B. Justice, Jr., Departments of Psychology and Neuroscience, University of Mississippi, MS 38677.

Place conditioning (PC) induced by intraperitoneal (IP) cocaine is considered to be mediated by a local anesthetic and central dopaminergic component (Spyral et al. 1982). In the present study, cocaine-methiodide, a quaternary salt of cocaine which shares certain pharmacological properties with cocaine but does not permeate the blood-brain barrier, was compared to cocaine for its ability to induce place conditioning when administered either IP or intracerebroventricularly (ICV). Using a completely balanced PC design, rats treated with IP cocaine (15 mg/kg) exhibited a significant preference for the drug-paired compartment on the test day (P<0.01). In contrast, rats receiving either cocaine-methiodide (5, 10, or 20 mg/kg) or propranolol (25 or 50 mg/kg) did not exhibit a preference. ICV infusions of cocaine (50 μg/2 μl) or cocaine-methiodide (5 or 10 μg/2 μl) produced significant place conditioning for the drug-paired compartment (P<0.05). In a separate experiment, ICV injections of cocaine (15 mg/kg) increased extracellular dopamine in the NACC, as measured by microdialysis. In addition to increasing locomotor activity while cocaine-methiodide failed to increase either dopamine or locomotor activity. These results suggest that PC induced by IP cocaine is mediated primarily by a central component. Furthermore, the local anesthetic effects produced by cocaine-methiodide and propranolol are not sufficient to induce PC with a balanced design.


In attempting to confirm our findings of decreased muscarinic and increased benzodiazepine binding at rat caudate nuclei thirty days after five daily exposure to continuous cocaine, behavioral challenges as well as additional biochemical tests were conducted. Rats were administered continuous cocaine, daily cocaine injections, cocaine injections without or after drug withdrawal. In a separate experiment, IP injections of cocaine (15 mg/kg) increased extracellular dopamine in the NACC, as measured by microdialysis. The potent reinforcing effects of cocaine can readily become associated with salient environmental stimuli, which then acquire secondary reinforcing properties. It has been proposed that the reinforcing properties of these conditioned stimuli (CSs) are due to the ability of the CSs to elicit similar neural events as the drug itself. Given the large body of evidence that implicates the mesolimbic dopaminergic pathway in the rewarding properties of cocaine, the present study was designed to determine whether stimuli paired with cocaine elicit similar neurochemical changes as cocaine, as assessed by in vivo brain microdialysis.

The administration of cocaine (10 mg/kg for 7 days) in association with a specific environment (CS+) produced significant conditioned locomotion in that environment. Rats that received cocaine in their home cage (CS-) did not exhibit increased locomotion on the test day, as compared to subjects that received saline in both settings.

When administered acutely, cocaine (10 mg/kg) produced a potent unconditioned effect on intravisual dopamine (80% of basal values) in the nucleus accumbens. In contrast to the ability of repeated pairing of cocaine with a specific environment to produce conditioned locomotion, there was a lack of evidence for coacquisition of conditioned neurochemical events. Specifically, the modest increase in dopamine (10%) above basal values) observed after exposure to the conditional environmental stimuli was equal in the CS+ and CS- groups. These data do not support the hypothesis that stimuli paired with cocaine produce their behavioral effects by eliciting similar neurochemical effects as cocaine.

Research revealed that the possible neurotoxicity of MDMA has caused renewed interest in the properties of another amphetamine derivative, fenfluramine (FFA), an anti-obesity drug. MDMA and FFA affect central and peripheral systems dependent on serotonin (5-HT). Both can reduce extracellular 5-HT levels for months. MDMA or FFA (0, 5, 10, or 20 mg/kg, s.c.) were used to compare 5-HT related endpoints, locomotor activity, body temperature and weight. Left male rats were dosed at 7 AM and 7 PM for 7 consecutive days. Body weights were obtained daily. At 0.5 hr following the 7 AM dose rectal temperatures were obtained and rats were placed in figure-8 mazes for measurement of locomotor activity. MDMA caused a biphasic activity pattern: the first 2 days and the 4th and 5th showed slight increases; 5 and 10 mg/kg showed decreases of 10% and 20% respectively. Conversely, FFA only produced dose-related decreases in locomotion. Toleration to the effects of either MDMA or FFA on activity did not occur with repeated administration. MDMA caused hyperthermia with incomplete tolerance to repeated dosing; temperature was still significantly elevated on the final day of dosing. FFA hyperthermia was evident at the end of daily maze testing; partial tolerance occurred to this effect. MDMA and FFA reduced body weight as well as food and water consumption during the period of dosing. Both control and treatment groups exhibited a 1.5-2.5% weight loss over the 7 day period. No body weight changes were observed in the control group, challenge with 1 mg/kg AMPH 30 min before sacrifice, elicited a 5-HT uptake inhibitor, fluoxetine (10 mg/kg), prior to each dose of MDMA. Rats were killed 2 days post dosing, brains were removed, and dorsal striatum was analyzed for 5-HT and 5-HIAA using HPLC. The 5-HT metabolite was not decreased at the 150 mg/kg dosage. MDMA increased GFAP by as much as 2-fold in specific regions of frontal and posterior cortex; the effect was graded with dose, detectable at 25 mg/kg, and did not alter 5-HT depletion. Fluoxetine antagonized the GFAP increase in frontal cortex but not posterior cortex. These data indicate that MDMA damages serotonergic and non-serotonergic targets in rat neocortex. SUPPORTED BY NIDA IAG ND-89-4.


MDMA (0,25,50,75,100,150 mg/kg) was administered to rats twice daily for 5 days and the animals were sacrificed by transcerebral perfusion with fixative under pentobarbital anesthesia 48 hrs postdosing. Additional rats receiving 100 mg/kg were sacrificed at various times after the second and fourth dosing. Frozen sections of the brain were stained with a recent modification of the de Olmos cupric silver stain for neuronal degeneration, GFAP immunoreactivity or cresyl violet. As previously reported, the de Olmos stain revealed evidence of terminal, axonal and perikaryal degeneration in the neocortex. Such degeneration was dose-dependent and detectable after 25 mg/kg. Degeneration was evident as early as 18 hrs after the second dose of 100 mg/kg and persisted for up to 14 days after the fourth dose. The regional pattern of degeneration did not coincide with known patterns of serotonergic innervation and was only partially blocked by fluoxetine implicating serotonergic as well as non-serotonergic targets. SUPPORTED BY NIDA/EPA IAG ND-89-4.


Repeated amphetamine (AMPH) treatment leads to a behavioral sensitization in rats characterized by increases in stereotyped and rotational behaviors. We have found that repeated AMPH increases the CaM-binding protein, calmodulin (CaM) in striatum (Roberts-Lewis et al., Brain Res., 384:383, 1986). To determine whether the AMPH-induced increase in CaM shares common characteristics of AMPH-induced sensitization, CaM was measured by RIA in cytosol and 100,000 x g membrane fractions in rat brain 30 min after a single dose of amphetamine to crowded mice did not appreciably affect brain DA and norepinephrine (NEA) and proteins may be region specific. Further, these results implicate serotonergic and non-serotonergic targets in rat neocortex.

567.6 METHAMPHETAMINE NEUROTOXICITY AND STRIATAL GLUTAMATE RELEASE. J.L. Yamamoto and J.P. Nash, Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, Ohio 44106.

Methamphetamine (MA) neurotoxicity is blocked by the excitatory amino acid antagonist MK801 (Bonnabry et al., 1986). The present study examined in vivo the effects of repeated injections of MA or the MA analog, 3,4-methylenedioxyamphetamine (MDMA) on extracellular levels of glutamate (GLU) and dopamine (DA) in the rat striatum. Male rats were implanted with 14C-glutamate microdialysis probes into the anteromedial caudate-putamen. Dialysate samples were collected every 30 min. Following a 1.5 hr stable baseline, vehicle or equivalent doses of MA (7.5 mg/kg) or MDMA (9.2 mg/kg) were injected i.p. 3 times every 2 hrs into awake rats. Dialysates were analyzed for amino acids and biogenic amines by HPLC/EC. One week later, rats were sacrificed and the caudate dissected and assayed for DA, DOPAC, and HVA. MA produced a cumulative elevation in extracellular GLU resulting in a 9-fold increase 2 hrs after the third injection. In contrast, MDMA produced a non-significant 1.8-fold increase. Each injection of MA or MDMA also produced an immediate and reproducible increase and subsequent decrease towards baseline in extracellular DA levels. DA and HVA tissue content and metabolites were decreased by 32% and 37% respectively, in MA but not MDMA-treated rats. No changes were detected in SHT or 5HTIA content. These results provide support for a GLU-mediated mechanism of MA neurotoxicity. These data also suggest that, in contrast to MA, MDMA toxicity may be mediated by a mechanism other than through an elevation in extracellular GLU.

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THURSDAY PM

DRUGS OF ABUSE—AMPHETAMINE

The biochemical mechanisms that regulate sensitization to amphetamine (AMPH) are not clearly defined although several studies have implicated corticosterol releasing hormone (CRH) as a possible component of the system. In the present study, female DBA mice were adrenalectomized (ADX) or sham operated with 80 mg hormone pellets containing 100% cholesterol, 100% progesterone or a 60% cortisol/40% progesterone mixture (CSS50). Following a 3 day recovery period, locomotor activity (number of photocell interrupts in 15 min) was measured in a circular open field arena, 15 min. following a saline injection or AMPH injection. Treatment groups did differ in activity following the saline challenge. On the 4 subsequent testing days, saline administration was continued for some animals while others received AMPH (5.0 mg/kg). CSS50 animals (SHAM and ADX) were initially more sensitive to AMPH than other treatment groups but did not develop behavioral sensitization. ADX animals were initially insensitive to AMPH but developed sensitization, although the magnitude was not the same as in sham-operated animals. Animals with progesterone implants were patterned of sensitization that were identical to control animals. CRH levels in the CSS50 animals are probably negligible due to enhanced negative feedback by high plasma GC levels that persist throughout the duration of testing. The data support the hypothesis that drug-induced changes in the hippocampal-pituitary-adrenal axis may underlie the development of behavioral sensitization to AMPH. Supported by DA-05151 and DA-00116.

567.8 THE NMDA RECEPTOR ANTAGONIST MK-801 BLOCKS THE DEVELOPMENT OF SENSITIZATION TO THE LOCOMOTOR ACTivating EFFECTS OF AMPHETAMINE IN THE RAT. E. Vidal and B. Zatorskis. Neurosciences, Lexi Research Institute, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9 Canada.

The NMDA receptor has been shown to be involved in long-term potentiation and kindling as well as in some types of learning and memory. In the present study, the possibility that this receptor might also play a role in the development of sensitization to the locomotor activating effects of amphetamine was examined. The ability of the NMDA receptor antagonist MK-801 to block its development was assessed. Different groups of rats were injected with either saline or MK-801 (0.3 mg/kg, i.p.) prior to each of five injections of AMPH (1.0 mg/kg, i.p.) given every third day. On a subsequent test for sensitization, all animals received saline followed by AMPH (0.3 mg/kg, i.p.). Animals' locomotor activity was monitored following all injections. MK-801 by itself produced dramatic increases in locomotion but, unlike those produced by amphetamine, these did not increase with repeated injections nor did they produce enhanced responding to amphetamine on the test for sensitization. More importantly, animals receiving MK-801 prior to each amphetamine injection showed no evidence of sensitized locomotion on the subsequent test. These results are consistent with those of earlier reports (Karler et al., Life Sci. 45: 599, 1989) and suggest that NMDA receptors play an important role in the development of sensitization to the locomotor effects of amphetamine. They also demonstrate that the repeated elicitation of increased locomotion by itself is not sufficient to produce sensitized locomotor responding.

567.9 ATTENUATION OF THE LOCOMOTOR ACTIVITY AND THE STRIATAL AND NUCLEUS ACCUMBENS DOPAMINERGIC RESPONSES TO AMPHETAMINE BY 3-METHYXISONITROXANE. M. S. Hooks, B. D. Luce, J. L. Uttaro, and S. G. Hoffer. Departments of Chemistry and Pharmacology, Emory University School of Medicine, Atlanta GA, 30322.

Naltroxone (NX), an opioid antagonist, has been shown previously to reduce behavioral effects of amphetamine (AMPH) in rats. The neurochemical basis of this interaction is unknown, but could be due to the ability of NX to attenuate the dopaminergic response to AMPH. The present study tested this possibility by determining the effects of NX (1 mg/kg, i.p.) on the locomotor responses to AMPH and 2) AMPH induced release of dopamine (DA) in the striatum (STR) and the nucleus accumbens (NACC). Male Sprague-Dawley rats were used in this study. In the first experiment, rats were initially treated with either NX (5 mg/kg) or saline and 30 min later received increasing cumulative doses of AMPH (0.01, 0.1, 1.0, 10.0, and 60.0 mg/kg) at 30 min intervals. Gross locomotor counts following AMPH injection were counted using a Behavioral Pattern Monitor (BPM) in which infrared photobeams tracked the behavior of the animal in a circular open field arena, 15 min. following a saline injection or AMPH injection. Treatment groups did differ in activity following the saline challenge. On the 4 subsequent testing days, saline administration was continued for some animals while others received AMPH (5.0 mg/kg). CSS50 animals (SHAM and ADX) were initially more sensitive to AMPH than other treatment groups but did not develop behavioral sensitization. ADX animals were initially insensitive to AMPH but developed sensitization, although the magnitude was not the same as in sham-operated animals. Animals with progesterone implants were patterned of sensitization that were identical to control animals. CRH levels in the CSS50 animals are probably negligible due to enhanced negative feedback by high plasma GC levels that persist throughout the duration of testing. The data support the hypothesis that drug-induced changes in the hippocampal-pituitary-adrenal axis may underlie the development of behavioral sensitization to AMPH. Supported by DA-05151 and DA-00116.


Repeated administration of opiate amphetamine (AMP) results in an augmentation of the locomotor stimulant effects elicited by subsequent AMPH challenge. The present study examined the possibility that this behavioral sensitization may require stimulation of N-methyl-D-aspartate (NMDA) receptors. Rats received injections of saline, AMPH (1 mg/kg), the NMDA antagonist MK-801 (0.25 mg/kg), AMPH + MK801 (0.25 mg/kg, i.p.) or saline + 0.25 mg/kg MK-801 each day for 10 days. Rats were tested for horizontal locomotor activity on several treatment days to verify that a progressive augmentation of responsiveness to AMPH was occurring. On day 14, all rats were challenged with 0.1 mg/kg AMPH and tested for locomotor activity. Sensitization was observed only in the repeated AMPH group, indicating that coadministration of MK-801 blocked the development of sensitization. On day 17, all rats were challenged with 0.25 mg/kg MK-801. The MK-801 and AMPH + MK801 groups showed robust sensitization, indicating that repeated MK801 pretreatment did not block the rat's own locomotor stimulant effects and that this was not blocked by AMPH coadministration. On day 13, all rats were challenge with saline. The AMPH group showed greater activity than the saline group, suggesting the development of conditioned locomotion. However, conditioned locomotion was blocked by coadministration of MK-801. These results suggest that behavioral sensitization may resemble long-term potentiation, a more thoroughly characterized form of synaptic plasticity, in that both require stimulation of N-methyl-D-aspartate (NMDA) receptors, show reward-related behaviors, and are blocked by MK-801. Further, the development of conditioned control of sensitization to AMPH and to its own locomotor stimulant effects that may require stimulation of NMDA receptors.
567.13

AMP/KAINATE GLUTAMATE RECEPTOR ANTAGONISTS IN THE NUCLEUS ACCUMBENS SELECTIVELY DECREASE THE HYPERMOTILITY RESPONSE TO AMPHETAMINE. D.L. Wallace, L.K. Uretsky, N.J. Uretsky, M.C. Gerald, and J.J. Wallace, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Previous studies have shown that activation of AMPA and/or kainate receptors in the NA play a role in the hypermotility response to the psychostimulant drugs, amphetamine, dopamine, and scopolamine. Both OFQX (1 μg/kg, i.p.), a chemical analog of DNQX which has very low potency for AMPA receptors, and GAMS (5 μl), a chemical analog of DNQX, inhibited the hypermotility responses to D-amphetamine (0.5 μg/kg, i.p.), but not to caffeine (20 μg/kg, s.c.). In contrast, AFOX, a chemical analog of DNQX which has very low potency for AMPA receptors, was ineffective. Neither A/K antagonist significantly affected LMA in control animals. These findings suggest that activation of A/K receptors in the NA is important in amphetamine-stimulated locomotion. Since the response to amphetamine, but not caffeine or scopolamine, is mediated by endogenous dopamine in the NA, the results suggest an association between glutamatergic and dopaminergic transmission at this site.

567.14

EFFECTS OF INTRA-ACCUMBENS INJECTION OF THE GLUTAMATE ANTAGONIST DNQX ON PLACE PREFERENCE AND LOCOMOTOR ACTIVITY INDUCED BY AMPHETAMINE. T. U. Seki, Y. N. Seki, L.K. Uretsky, and J.J. Wallace, College of Pharmacy, Ohio State University, Columbus, OH 43210.

Dopaminergic terminals within the nucleus accumbens (NA) mediate locomotor activity (LMA) and positive reinforcement elicited by amphetamine. Glutamatergic afferents to the NA from limbic structures may also participate in these effects. The purpose of this study was to determine whether an AMPA/kainate receptor antagonist within the NA could inhibit amphetamine-induced LMA or reinforcement as measured by conditioned place preference (CPP). Rats were trained for 8 days, with 4 amphetamine pairings (1 mg/kg, s.c.) to one compartment and 4 saline pairings to the other. One group of rats received DNQX (1 μg/kg, i.v.) in the NA via implanted cannulae at the time of amphetamine injections. Rats were tested for CPP on the 9th day. LMA was monitored on all training and test days. Consistent with data from open field tests, DNQX inhibited amphetamine-induced LMA by 60% during the first training session. However, DNQX reduced LMA by only 20% on the 2nd amphetamine training day and not at all in the final two training sessions. Thus, the interaction between dopaminergic and glutamatergic neurons on the reward with exposure to amphetamine. This confounding variable makes interpretation of CPP data difficult, although preliminary data suggest that DNQX treatment inhibits amphetamine-induced CPP.

567.15

MK-801 PARTIALLY ANTAGONIZES THE LOCOMOTOR AND STEREOTYPIC EFFECTS OF METHAMPHETAMINE. G.M. Partie, N.E. Ferguson and L.S. Schedes, Department of Pharmacological & Physiological Sciences, The University of Chicago, Chicago, IL 60637.

MK-801, a non-competitive NMDA receptor antagonist, has been shown to attenuate both amphetamine- and cocaine-induced hyperactivity (Koob, et al., Life Sci., 45:599-606). To determine whether MK-801 (MK) antagonizes behaviors elicited by a neurotoxic regimen of deprenyl (10 mg/kg, s.c.), rats were studied in a 4-choice scale with exposure to amphetamine. This confounding variable makes interpretation of CPP data difficult, although preliminary data suggest that DNQX treatment inhibits amphetamine-induced CPP.

567.16


Squirrel monkeys' low-rate punished lever pressing (food-reinforced fixed-interval schedule with superimposed fixed-ratio shock) is reliably unaffected or decreased by d-amphetamine (AMP), AMP + cocaine (COCA), or scopolamine, is mediated by endogenous dopamine in the NA, the results suggest an association between glutamatergic and dopaminergic transmission at this site.

The recent discovery of the abuse of methamphetamine (METH) in a smokable form known as "crank" or "crack" suggests that drug abuse may be increasing. As such, it is important to investigate the potential toxic consequences of its abuse. We studied the effects of METH on cardiovascular function in squirrel monkeys, a model we have used extensively to study the cardiovascular effects of cocaine. Squirrel monkeys were implanted with chronic arterial and venous catheters and the effects of METH stimulation and withdrawal of stimulation on blood pressure and heart rate were measured. METH (0.1-3.0 mg/kg, i.v.) produced a dose-dependent increase in blood pressure. Its effects on heart rate were more complex, with lower doses (0.1-0.3 mg/kg) producing increases in heart rate and higher doses (1.0-3.0 mg/kg) producing decreases. To determine the pharmacological mechanisms involved in METH's effects, we used a number of drugs as pretreatments to an injection of 0.2 mg/kg METH. This dose produced a maximal heart rate increase. The alpha-1 agonist prazosin (0.1 mg/kg, i.v.) completely antagonized the effects of METH on blood pressure, while the beta antagonist propranolol (3.0 mg/kg, i.v.) completely antagonized the tachycardia effect of METH. Propranolol also appeared to potentiate the effects of METH on blood pressure by prolonging its effect. These effects are similar to those observed previously with prazosin and propranolol when tested against cocaine (FASEB J. 5, A157). Unlike its effects against cocaine (Life Sci. 48, 1547), however, the dopaminergic antagonist haloperidol (0.03 mg/kg, i.m.) antagonized both the pressor and tachycardia effects of METH. Thus, while METH and cocaine produce similar effects on cardiovascular function, they may be mediated through different mechanisms, with dopaminergic mechanisms being more important for METH than for cocaine.

567.19

ETHANOL REGULATES TYROSINE HYDROXYLASE GENE EXPRESSION IN N1E-115 NEUROBLASTOMA CELLS, Greg G. Gayer*,†, Adrienne Gorden† and Michael F. Miles†. Ernest Gallo Research Center and, Departments of Pharmacology and Cell Biology, Univ. of Calif., San Francisco, CA 94134. Previous studies show that changes in catecholamines contribute to neurophysiologic effects of ethanol. We have therefore studied ethanol-induced changes in tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine biosynthesis, in N1E-115 neuroblastoma cells. Northern and Western blot analyses showed that a 3 day treatment with 25-200 mM ethanol caused a dose-dependent increase in TH-mRNA and protein levels in N1E-115 cells. Subclones of N1E-115 stably transfected with a plasmid containing the TH promoter fused to a chloramphenicol acetyltransferase (CAT) reporter gene showed ethanol-induced increases in CAT activity. These data suggest that ethanol modulates TH gene transcription. Transient treatment of transfected cells with 100 mM ethanol and either 1 mM prostaglandin E1, 10 nM (-)-N6-(R-phenylisopropyl)-adenosine, or 0.5 mM 8-bromo-cAMP, potentiated TH-promoter activity when compared to treatment with the respective agents without ethanol. These results suggest that chronic ethanol has a prominent effect on both basal and cAMP-regulated TH expression. Using deletion analysis of the cis-acting regulatory sequences of the TH-promoter, the regions responsible for both basal and cAMP-mediated ethanol induction were characterized. Ethanol-induced changes in TH expression may be a critical aspect of central nervous system adaptation to chronic ethanol exposure.

568.1

mRNA EXPRESSION PROFILES AFTER OPIATE TREATMENT IN CELL CULTURE & THE RAT CNS. SA Mackler & JH Eberwine. Dept. of Pharmacology, Univ. of Pa School of Medicine, Philadelphia, PA 19104.

Opiate use alters the amounts of several proteins. The present studies use the in vitro amplification of RNA from limited amounts of tissue (aRNA;PNAS87:16635) to define the composite of relative changes in mRNA abundance which result from opiate stimulation or withdrawal of stimulation.

NG108-15 cells were exposed to 10nM DADLE or 10nM DADLE followed by 100nM naloxone for varying time periods to achieve maximal delta opiate receptor down- and up-regulation, respectively. Polya+ RNA was isolated and synthesized into 32-P labeled aRNA. This was hybridized to blots containing several cDNA clones and comparisons made among the individual autoradiographic bands. Both receptor stimulation & withdrawal of stimulation resulted in increased signals for the pre-pro-opioid peptides Ss and Ds and decreased signals for Kdelta channels. Naloxone increased the signal for the Na+ channel & DADLE depressant stimulation & withdrawal of stimulation resulted in increased signals for the Na+ channel & increased the signals for fos, jun, & Gs alpha. These studies demonstrate several changes in gene expression as a consequence of opiate use and provide hypotheses for testing the molecular mechanisms of addiction.

568.2

LONG TERM EFFECT OF PRENATAL METHADONE EXPOSURE ON NEUROPEPTIDE EXPRESSION IN RAT HYPOTHALAMUS. J. W. Nemitz. W VA School of Osteopathic Medicine, Lewisburg, W VA 24901.

Previous work provided evidence that prenatal methadone exposure results in decreases in mRNA expression for neuropeptides (ENK), oxytocin (OT) and arginine-vasopressin (AVP) in hypothalamic nuclei of the neonatal rat. The present study examined the mRNA expression of these neuropeptides in a group of older rats that were prenatally exposed to methadone.

Adult, female, Sprague-Dawley rats made physically dependent to methadone (9mg/kg/day) by an osmotic minipump (Alzet), were mated and the pregnancies brought to term. Offspring were sacrificed by decapitation after six months to one year after birth. Frozen serial coronal sections (20 um) obtained at the level of the hypothalamus were processed for in situ hybridization using [35S]-labeled nucleic acid probes (DuPont). Results indicate that OT and AVP mRNA levels are increased in the retrochiasmatic supraoptic nucleus (RSo) of the hypothalamus of prenatally methadone exposed rats as compared to controls. Differences were not observed in the paraventricular (PV) and supraoptic (SO) nuclei. Results for ENK mRNA expression will also be presented. These data suggest that prenatal methadone exposure can result in selective long lasting changes in neuropeptide expression for a hypothalamic area.

568.3

EFFECTS OF PRENATAL MORPHINE ON ADULT SEXUAL BEHAVIOR AND ON BRAIN CATECHOLAMINES IN RATS. Ilona Vathy. Dept. Psychiatry, Albert Einstein Coll. Medicine, Bronx, NY 10461.

Female rats exposed to morphine sulphate (MS) in utero (5-10 mg/kg twice a day on days 11-18 of gestation) were substantially inhibited in their adult sexual behavior when compared to saline-exposed (S) controls. In contrast, males exposed prenatally to MS had shorter post-ejaculatory intromission latencies and exhibited increased mounting and intromittion activity relative to controls. Examination of the catecholamine content in the prooptic area (POA) and in the hypothalamus (HYP) also revealed that prenatal MS treatment affects males and females differently. Noradrenaline (NE) and dopamine (DA) content in the HYP of S and MS-treated females were essentially identical; however, NE and DA levels in the POA of MS-treated females were decreased about 60%. NE content in the HYP of MS-treated males was increased about 50% relative to that in S-exposed animals while the DA content was not affected. In addition, the DA content was decreased (30%) and the NE content increased (30%) in the POA of MS males when compared to controls. These results suggest that prenatal MS exposure, which differentially affects adult male and female sexual behavior, also alters the content of catecholamines in the HYP and POA in a sexually dimorphic fashion. Supported by NIDA DA 05833.
568.5  CP55940 HAS THC-LIKE DISCRIMINATIVE STIMULUS EFFECTS IN RATS. L.H. Gold, R.L. Barrett, R.L. Balster and B.R. Martin*. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298-0611. CP55940, a phenyl-cyclohexanol derivative, is a potent bicyclic analog of 9-tetrahydrocannabinol (THC) which has been used as a probe for a cannabinoid recognition site in neural tissue. In the present study, CP55940 was evaluated in rats trained to discriminate THC (3.0 mg/kg, ip) from vehicle in a two-lever operant procedure. In addition, catalepsy was measured using an adaptation of the mouse-ribbon test. Dose-effect relationships were established for the training drug (THC: 0.01-10.0 mg/kg, sc) and CP55940 (0.003-0.3 mg/kg, ip) at 30 and 90 min after injection. CP55940 substituted completely for THC at both time periods at a dose (0.1 mg/kg) that had minimal effects on rate of responding. CP55940 was about 10-30 fold more potent than THC, consistent with its greater affinity for the cannabinoid site. Doses of THC (0.3 mg/kg) and CP55940 (0.1 mg/kg) that reduced response rates by greater than 50% were associated with substantial increases in immobility. A study of the duration of drug effects revealed that both CP55940 (0.1 mg/kg) and THC (3.0 mg/kg) produced diminished levels of THC-lever responding by 360 min post-injection. These results support the use of CP55940 as a probe for the neural substrate mediating the behavioral responses to THC. (Supported by NIDA grants DA-03672 and DA-07027.)

568.7  TETRAHYDOCANNABINOL EFFECTS ON BRAIN STIMULATION REWARD AFTER SUBACUTE HALOPERIDOL OR AMPHETAMINE. M. Borreto and R. Nune. U. of Puerto Rico Sch. Pharm., San Juan, PR 00936. Using a brain stimulation reward (BSR) procedure that has demonstrated threshold decreases and response rate increases after low morphine doses (Psychopharmac. 102:309, 1990), we have found non-iform changes in BSR thresholds after low doses of Δ9-tetrahydrocannabinol (THC) in Lewis and SD rats (The FASEB Journal 5: A703, 1991). Since the BSR threshold lowering effect of THC only in Lewis rats, reported by Gardner, has been related to facilitation of mesolimbic dopaminergic (DA) release by THC (Psychopharmac. 96: 142, 1988; 102: 156, 1990), we tested the hypothesis that THC would lower BSR thresholds, and/or increase response rates, in SD rats that were sensitized to DA agonists. Twelve days after withdrawal from osmotic pump infusion of haloperidol (0.4 mg/rat/day for 14 days), significant increases in BSR rates occurred in male SD rats, and this treatment attenuated the threshold increases seen after 0.5 and 1.0 mg/kg THC doses; THC-induced threshold decreases were not seen at other doses, nor did they occur reliably in female SD rats receiving similar THC challenge doses 11 days after withdrawal from a week of daily d-amphetamine sulfate injections, a procedure reported to increase mesolimbic DA utilization in female rats (Robinson: Brain Res. 343: 374, 1985). The basis for the reported strain-specific BSR threshold lowering effect of THC is not apparent and requires further investigation to clarify the conditions that permit unequivocal designation of THC as a rewarding drug. Supported by 50688824.

568.9  MORPHINE REDUCES THE AVERSIVENESS OF NOVEL QUININE SOLUTION. L.A. Parker, Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario, Canada, N2K 3C5. The ability of morphine (2 mg/kg, sc) to modify the palatability of 0.5% quinine solution and 20% sucrose solution was assessed in Sprague-Dawley rats using the Taste Reactivity (TR) test. The TR test is a direct measure of the palatability of tastants. Morphine suppressed the aversive TR responses of chin rubbing, gaping and paw treading elicited by quinine on the first 10 minutes trial without modifying general activity level (horizontal and vertical). The effects of morphine on the palatability of sucrose solution were minimal.

568.10  ANABOLIC STEROIDS DISRUPT LATENT INHIBITION. M.J. Kelley*, R.B. Messing1, E. Nwokolo1 and S.B. Sparber1. 1Pharmacology Dept., 2Univ. of Minnesota, Minn. Dept. Health, Mpls. MN 55455. Latent inhibition (LI) was studied in male rats by exposures to a Skinner box prior to administering grid shock in the box. Control rats were exposed to a nesting cage followed by shock in the Skinner box. Conditioned-freezing behavior 24-hr after shock was the dependent variable. In the first experiment, testosterone (13.5 mg/kg), or an equimolar dose of nandrolone or olive-oil vehicle were administered seven times over 17 days, prior to and concurrent with the LI procedure (N = 36). A second multi-dose experiment assessed LI 10-months after the administration of anabolic steroids during "adolescence" (N = 73). Both experiments showed a LR effect with vehicle (p < .01) but not the testosterone groups. Nandrolone administered 10-months earlier also disrupted LI but had a marginal effect when concurrent with LI testing. Androgenic (testosterone and nandrolone) and estrogenic (metabolism of testosterone) effects may be differentially involved in acute versus enduring influences on LI. Supported in part by USPHS grant DA011979 and MMF grant SM68-90.

The role of in o situ analgesia remains unclear. Intraperitoneal (IP) administration of GABAergic drugs was found to potentiate morphine analgesia, while intracerebroventricular (ICV) injections of morphine into the nora- phine-sensitive CNS site, the periaqueductal gray (PAG), were reported to reduce morphine analgesia. The present study investigated GABA levels in dialysates obtained from the rat PAG during morphine administration. A microdialysis probe (BAS) was inserted into chloral hydrate anesthetized male rats (B.W. approx. 230 g) mounted on a Kopf stereotaxic. Dialyates were collected in 30-min samples (after discarding the first 60-min dialysate) during perfusion of Ringers at 1 μl/min. Morphine was given IP (0.1-5.6 mg/kg) and ICV (0.1-5.6 mg/kg) through the probe following a 2- to 4-hr period, and collection continued for 1.5 hrs. GABA was evaluated using the BAS HPLC electrochemical detection method. All rats brains were examined histologically to determine probe placement. Decreases in GABA levels were found when probes were located within the PAG, while more variable results were seen in other sites.


The potential antagonist action of d-serine, a serine analogue for the glycine/NMDA receptor, against the EEG and behavioral effects of PCP, a noncompetitive NMDA antagonist, was investigated. In rats bearing chronic cortical EEG electrodes and iv and icv cannulae PCP (1.25 mg/kg, iv) produced a behavioral syndrome characterized by head weaving, locomotion, and ataxia. Five min pretreatment with d-serine (1 μM, icv) tended to reduce these PCP-induced behaviors, but the effect was not significant, nor dose-dependent (2 μM). Simultaneous recording of EEG frequency spectra revealed a PCP-induced dualpeak response centered about 2-3 Hz and 7-8 Hz which was also unaltered by d-serine. d-serine alone produced no significant EEG or behavioral changes. In rats trained to discriminate 1.2 mg/kg PCP ip versus saline on a FR 20 schedule of food presentation, PCP produced 93% responding on the PCP-correlated lever. This effect was mimicked by d-serine but not significantly altered by a two min pretreatment with d-serine (0.5-2 μM, icv). Collectively, these results fail to support a PCP antagonist effect by d-serine. Confirmatory icv PCP studies are in progress. (Supported in part by grant DA03173)

568.13 AN INTERACTION BETWEEN OPIOID AND NORADRENERGIC SYSTEMS IN THE MODULATION OF PULSATILE LUTEINIZING HORMONE SECRETION BY DELTA-9-TETRAHYDROCANNABINOL. L.L. Murphy, P. Weinstock and M. Kohli. Department of Physiology, Southern Illinois University, School of Medicine, Carbondale IL, 62901.

We have previously demonstrated that naloxone (NAL), an opiate receptor blocker, can reverse the ability of delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, to inhibit pulsatile luteinizing hormone (LH) secretion in the ovariectomized female rat. Because the effect of endogenous LH releasing hormone (LHRH) secretion and, thus, LH release, may be mediated through hypothalamic noradrenergic systems, we attempted to block the NAL reversal of THC-induced LH inhibition by pretreatment with norepinephrine receptor blockers. Approximately 4 weeks postovariectomy, blood samples were drawn from adult Sprague-Dawley rats, via indwelling intra-atrial cannulae, every 10 min for 60 min pre- and 120 min post-vehicle (control) or THC (0.5 mg/kg, b.w., iv) treatment at time 0 for LH determinations. Either the β-receptor antagonist, yohimbine (YOH, 0.5 mg/kg, iv), or the α-receptor antagonist, prazosin (PRZ, 0.5 mg/kg, iv), was administered 30 min prior to THC or its vehicle, and 50 min before NAL (2 mg/kg, b.w., iv) treatment. Neither YOH nor PRZ altered pulsatile LH secretion in vehicle-exposed animals treated with NAL, and YOH pretreatment did not alter the ability of NAL to reverse THC action. However, pretreatment with PRZ prevented NAL reversal of THC-induced suppression of pulsatile LH release. These results suggest that THCaugment hypothalamic opioid activity which, in turn, could inhibit noradrenergic stimulatory inputs onto the LHRH secreting neurons. (Supported by DA 09492)

568.14 EFFECTS OF ANALGESIC DOSES OF MORPHINE ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. H. Zhang*, E.A. Stein and T.C. Trusk. Departments of Psychiatry and Cell Biology, College of Medicine, University of Iowa, Iowa City, IA, 52242.

The widespread distribution of opioid receptors and peptides in the central nervous system suggests an important role for this system in numerous brain functions including analgesia and regulation of numerous physiologic parameters. Included among these functions may be modulation of neurotransmitter systems, in particular the noradrenergic, which controls blood flow. The present study investigated the effects of morphine on neuronal activity as reflected by alterations in regional cerebral blood flow (CBF) using microdialysis probe (BAS) and quantitative autoradiography in awake, restrained rats. Morphine was delivered subcutaneously (2, 4 mg/kg) and measured CBF at 15 min later. Tail flick latencies increased at 2 mg/kg to 47% MPE and 100% at 4 mg/kg. The results showed that morphine caused a global dose-dependent increase in CBF in 39/51 structures analyzed. Mean change in CBF at 2 and 4 mg/kg were 27% and 71% respectively. At 4 mg/kg, morphine resulted in an increase in PaCO2 of approximately 6 mm Hg, while not changing PaO2, heart rate or blood pressure. Naloxone (10 mg/kg) was ineffective in changing either CBF or physologic parameters when delivered alone but was able to completely reverse the effects of 4 mg/kg morphine. These results suggest that opioid receptor stimulation by morphine may increase both functional neuronal activity as well as influence an intrinsic central neurogenic control of the cerebral circulation as well. (Supported by NIDA grant DA 05012)

568.15 RAPID DETERMINATION OF DRUG DISCRIMINATIVE STIMULUS EFFECTS UNDER A CTA PROCEDURE. T.M. Richardson*, E.A. Walker and A.M. Young. Psychology Dept., Wayne State University, Detroit MI 48202.

Conditioned taste aversion (CTA) procedures allow efficient characterization of discriminative stimulus effects of drugs. This study developed a method for generating cumulative dose-response curves (DRCs) using multiple trial tests. Rats (n=8) were trained to discriminate 3.2 mg/kg morphine (MS) and saline (SAL) by giving either MS or SAL 15 min prior to 30-min access to a 0.1% saccharin solution, followed by post-injection sessions of 75 mg/kg LC3 or SAL, respectively. A control group (n=4) received identical pre-injection, but always received SAL post-injection. Five subjects from each group received single trial tests in which an acute dose of a drug was given 15 min prior to 30-min access to saccharin. Generalization was measured by differences in the dose needed to suppress drinking in the conditioned and unconditioned groups. MS tested at doses of 0.1-10 mg/kg and buprenorphine (0.007-0.1 mg/kg) evoked dose-dependent generalization with MS, whereas ketamine (0.1-32 mg/kg) suppressed drinking at identical doses in both groups. Three subjects from each group received multiple trial tests, in which 5 trials per session were given. Multiple trial tests generated DRCs similar with those from single trial tests. MS (0.1-10 mg/kg), buprenorphine (0.01-0.32 mg/kg) and naltrexone (0.032-32 mg/kg) evoked generalization with MS, whereas ketamine (0.1-32 mg/kg), U50488H (0.1-5.6 mg/kg) and naltrexone (0.1-32 mg/kg) did not. In conclusion, multiple trial testing in the CTA procedure allows rapid, pharmacologically-specific evaluation of discriminative stimulus effects of drugs. (APA Minority Fellowship Program and by USDA grants DA05976 and K20-DA00132).

The effects of long-term lithium treatment on extracellular NA, DA, and 5-HT was examined in the rat medial prefrontal cortex using the in vivo brain microdissection method. Potassium (K*)-induced the perfusion rate induced substantial increases in NA, DA and 5-HT outputs in a concentration-dependent manner within the range of K* (0.1-3mM).

Lithium maintained a decrease in the levels of the efflux of potassium (K*) for 7 days resulted in significant increases in the K* (60mM)-induced NA, DA and 5-HT release.

These results suggest that lithium treatment enhances the activity of presynaptic monoaminergic neurons.

LITHIUM INCREASED NEURAL EXCITABILITY WITHOUT CHANGING ENDOGENOUS LEVELS OF MONOAMINES IN RAT HIPPOCAMPAL SLICES STUDY. J.-C. Lacaille, S. Cloutier*, F. Lebel* and T.A. Reader.

J.-C. Lacaille, S. Cloutier*, F. Lebel* and T.A. Reader.

In rats, downregulation of β adrenergic receptors after chronic administration of antidepressants (ADs) has been well documented. Both β1 and β2 receptors have been shown to produce this effect. In addition, 5-HT2 receptor downregulation has been demonstrated after chronic treatment with antidepressants (ADs). The results are inconsistent for 5-HT1D receptor blockers. To date, no reports are available on the effects of 5-HT1D receptor blockade on the regulation of 5-HT1D receptors.

Inaima et al. showed that mifepristone decreased corticosterone levels. In the present study, we investigated the acute effects of nefazodone, which interacts with both 5-HT uptake and the concentration of HVA, DOPAC, 3-MT and 5-HT in the brain of rats.

Taken together these results are suggestive that lithium treatment enhances the activity of presynaptic monoaminergic neurons.

Lithium is an effective mood-stabilizing agent. To study its mechanism of action, we have examined the acute effects of lithium (Li*), choline (Ch*), and sodium (Na*) on the release of monoamines in the brain of rats. In this study, the effects of Li*, Ch*, and Na* on the dose-dependent increase in pSPike amplitude was monitored in the pyramidal cell layer of the CA1 region prior to and after 20 minutes of superfusion with Li*, Ch* or Na* (1-30 mM). These data suggest that specific acute effects of lithium on neuronal excitability but not on endogenous levels of monoamines.
569.8

IMPRIAPINE BINDING AND EFFECTS OF REPEATED TREATMENT IN ADULT FEMALE RATS. S. Maywood, J. Williams and L. Urbouse. Department of Biology, Texas Woman's University, Denton, Texas, 76204

Although imipramine is one of the most widely investigated antidepressants, experimental studies have been performed primarily in the male rodent. However, in the human population, females are the major consumers of antidepressants and it was initiated to examine the effects of imipramine in a female rat with the ultimate objective of comparing these findings to those reported for the male population. In the first study, the effects of plus and minus desipramine as a competitor, to frontal cortex of estrous, proestrous and diestrous rats was examined. In general, binding profiles resembled those reported for males. However, the Kd for desipramine binding in the female frontal cortex was larger than reported for the male and, although not statistically significant, the Kd and Bmax of estrous rats tended to be lower than that of proestrous and diestrous rats. In the second experiment, female rats were injected i.p. with 10 mg/kg imipramine or saline daily for 21 days. Such treatment regimens are often employed in male rats in order to ascertain the neurochemical changes responsible for the drug's therapeutic action, which require 2-3 weeks. Disruption of the estrous cycle occurred rapidly following imipramine treatment. During the 21 days of treatment, most of the saline-treated rats had 4 consecutive estrous cycles. In contrast, imipramine-treated rats showed only 2 episodes of vaginal estrus, indicative of disruption of estrous cyclicity. These results raise the possibility that the alteration of the reproductive cycle could occur following treatment with other antidepressants.

569.9

SEROTONIN FUNCTION AND THE MECHANISM OF ANTIDEPRESSANT ACTION. P.L. Delgado, H.L. Miller*, R.S. Salomon*, J. Lopic, J.J. Plos, G.S. Hardinger, J.K. Whiteside, O.S. Charness, West Haven V.A.M.C. and Dept. of Psychiatry, Yale University School of Medicine, New Haven, Ct. 06509

Brain serotonin (5-HT) content is dependent on plasma levels of the essential amino acid, tryptophan (TRP). We have previously reported that rapid TRP depletion more frequently reversed antidepressant response to monoamine oxidase inhibitors and 5-HT reuptake inhibitors than to desipramine (DMI). This study was further intended to examine the relationship between TRP depletion and 5-HT availability to determine the extent to which TRP depletion may reverse antidepressant activity in non-refractory depressed patients randomly assigned to treatment with either DMI or fluoxetine (FLU). METHOD: In an ongoing study, 25 drug-free depressed (DSM-III-R) patients were randomly assigned to receive antidepressant treatment with either desipramine or fluoxetine. All patients were either treatment naive (N=15) or had had previous unsuccessful antidepressant treatment (N=10). 19 of the 25 patients had therapeutic responses by predetermined criteria to antidepressant treatment (DMI 710; FLU 1215) and 16 (6 DMI responders and 10 FLU responders) went on to TRP depletion testing. TRP depletion testing involved two 2-day tests, a 16-amino acid drink and a follow-up day, in a double-blind, placebo-controlled (TRP depletion and control testing), crossover fashion. On one test the drink was supplemented with 1 mg TRP (control) and on the other test the TRP was added (TRP depletion). Behavioral ratings (Hamilton Depression Scale (HDS)) and plasma (for TRP levels) were obtained prior to, during and after TRP depletion. Results: Total and free TRP decreased 70-80% 5 hrs. after the TRP-free drink. While 5/10 FLU responders relapsed, none of the DMI responders relapsed (one DMI-treated male responder). Tandospirone administered before training produced deficits in retention at test 1, 3 and 7 days later. This effect was reversed by d-amphetamine before test, supporting the hypothesis that retrieval, not learning, is effected by 5HT1A receptor activation.

569.10

MAZINDOL IN TREATMENT-REFRACTORY DEPRESSION. H.L. Miller*, S.M. Stige, P.L. Delgado, R.M. Salomon* and D.S. Charney, West Haven V.A.M.C. and Dept. of Psychiatry, Yale University School of Medicine, New Haven, Ct. 06508

Mazindol is a potent dopamine and noradrenaline reserapte, inhibitor which has previously been used for antidepressant activity in humans. This study examines the efficacy of mazindol in patients with treatment-refractory major depression. METHOD: 7 depressed patients (4 males and 3 females, ages 34-68) received placebo for the first two weeks of the study, then were treated with mazindol, at doses ranging from 1-4 mg, for a total of 16 weeks. The study was single-blind. Severity of depression was measured weekly with the 25-item Hamilton Depression Rating Scale (HDS). All 7 patients continued to be depressed throughout the placebo period and proceeded to active treatment. RESULTS: 2 of the 7 patients (29%) treated to date had a therapeutic response to mazindol (2, 50% decrease in HDSR and total ≤ 10). The drug was well tolerated; common side effects included insomnia, headaches, and urinary hesitation. Conclusions: 30% of this treatment-refractory population improved with mazindol treatment. Mazindol may have greater antidepressant efficacy in patients less refractory to treatment.

Somatodendritic 5-HT, autoreceptors are thought to play an important role in regulating the discharge activity of central 5-HT neurons. Recently, we have shown that blockade of the 5-HT autoreceptor with systemic apomorphine produces a reduction in the firing rate of 5-HT dorsal raphe nucleus (DRN) neurons in awake cats, suggesting that 5-HT autoreceptors exert a tonic inhibitory influence on the activity of 5-HT neurons under physiological conditions. The present study examines the effects of three other putative 5-HT, antagonists (BMY-7378, NAN-190, and (-)-propranolol) on DRN unit activity. 5-HT neurons were recorded and identified using methods previously described (Formal et al., Am. J. Physiol. 259:R963-R972, 1990). Drugs were administered via a jugular catheter. In contrast to the effects of apomorphine (0.25-1 mg/kg), intravenous administration of BMY-7378 (0.02-0.1 mg/kg), NAN-190 (0.02-1 mg/kg), and (-)-propranolol (1-3 mg/kg) suppressed the activity of DRN neurons and failed to block the inhibitory action of 8-OH-DPAT, a selective 5-HT, agonist. In addition, the suppression of DRN unit activity produced by BMY-7378, NAN-190, and (-)-propranolol was antagonized by apomorphine. These results indicate that these putative 5-HT, antagonists act as agonists rather than antagonists at the 5-HT, autoreceptor site.


Ascending serotonergic projections from the midbrain raphe nuclei have been implicated in the regulation of hippocampal electrical activity. Previous studies have shown that serotonin derived from the midbrain raphe nuclei inhibit the activity of hippocampal pyramidal cells. Recently, we have found a subgroup of serotonergic neurons whose activity appears to be inversely correlated with the occurrence of hippocampal RSA during waking behaviors. These findings have prompted us to examine the effects of 5-HT, autoreceptor agonists (8-OH-DPAT and ipsapirone) on hippocampal RSA in freely moving cats. Intravenous administration of low doses of 8-OH-DPAT (10-20 μg/kg) and ipsapirone (60-100 μg/kg) induced clear RSA in the hippocampal EEG. The onset of the effect (~20 sec) coincided with a general suppression of serotonergic unit activity recorded in the midbrain raphe nuclei. These data suggest that preferential activation of somatodendritic 5-HT, autoreceptors produces RSA in cats and support the hypothesis that central serotonergic neurons inhibit the generation of hippocampal RSA.


A-75200, ([1',7',9'-trimethyl-2',3',4',5'-tetrahydro-5'-methylene-dioxo-1-naphthalenyl] methyl) pyrrolidin methane sulfonate, is a potent, selective and efficacious inhibitor of norepinephrine reuptake. Because this unique profile suggests potential antidepressant activity, A-75200 was evaluated in an antidepressant screening procedure using olfactory bulbectomized (OB) rats. Sham operated (SO) rats were placed in activity monitors for 11 days on a 13 hour light - 11 hour dark cycle. OB rats were markedly more active than SO rats during the dark phase. Six injections of A-75200 (10.0 mg/kg, i.p.) once daily reduced the dark phase activity of OB rats to a level equal to that of SO rats. Imipramine (10.0 mg/kg, i.p., once daily) was effective after 8 injections. A-75200 did not affect locomotor activity, seizure threshold or nociception, and showed weak activity in the thisty rat conflict test. The efficacy of A-75200 in the OB rat supports its potential for antidepressant activity.


Our previous studies suggested that the sensitivity of the 5-HT, autoreceptor may vary across different raphe cell groups. The present study examines the effects of 5-HT, agonists and antagonists on unit activity in the dorsal raphe nucleus (DRN) and nucleus raphe centralis superior (NCS) in behaving cats (methods described in preceding abstract). Intravenous administration of the selective 5-HT, agonists 8-OH-DPAT and ipsapirone produced a dose-dependent inhibition of both DRN and NCS unit activity. However, 8-OH-DPAT and ipsapirone were more potent in suppressing the activity of DRN neurons (ED50 for 8-OH-DPAT = 1.2 μg/kg; ED50 for ipsapirone = 4.6 μg/kg) than NCS neurons (ED50 for 8-OH-DPAT = 3.0 μg/kg; ED50 for ipsapirone = 23.8 μg/kg). The 5-HT, autoreceptor antagonist apomorphine (1 mg/kg) produced a marked, and similar, increase in the firing rate of both DRN and NCS neurons. Overall, these results provide evidence for a differential sensitivity of DRN and NCS neurons to 5-HT, agonists and suggest that 5-HT neurons in both raphe nuclei are under tonic feedback inhibition.
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Retroviral vectors can selectively deliver "transgenes" to tumor cells. In culture, cells bearing the HSV-TK gene under the MoMLV LTR (C Ezzeddine et al., New Biol., 1991). To increase the efficiency of delivery of the HSV-TK gene to tumor cells, we infected C6 mouse neuroblastoma cells, support the conclusion that this promoter is active in neural crest-derived tissues, and is expressed at high levels in carcinomas. The recent development of herpes Simplex Virus (HSV-1) vectors has provided a useful method of stable gene transfer into the central nervous system (CNS), peripheral nerve cells, and muscle. The HSV-1 vector is a member of the herpesvirus family and has a high degree of homology to the human herpes simplex virus type 1 (HSV-1). The HSV-1 vector can infect both dividing and non-dividing cells, and can be used to introduce foreign genes into the mammalian genome. The HSV-1 vector has been used extensively in gene therapy applications, including the treatment of genetic disorders such as cancer and nerve damage. The HSV-1 vector has also been used in the delivery of therapeutic genes to the nervous system, including the treatment of neurological disorders such as Parkinson's disease and Alzheimer's disease. The HSV-1 vector is a powerful tool for the delivery of therapeutic genes to the nervous system, and has the potential to revolutionize the treatment of neurological disorders.
570.7 A POTENTIAL NEW METHOD OF DELIVERY OF AN EXPRESSED GENE TO CENTRAL NEURONS OF THE ADULT RAT

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A number of methods have been used to deliver expressible genes to cells. Viral vectors are relatively new but promise several advantages over other techniques. Advantages of viral vectors include high transduction efficiency, high yield of recombinant virus, and a large variety of host range and tissue selectivity. Viral vectors can be adapted for delivery of genes to the central nervous system by using neuronal vectors, which infect neural progenitor cells and can be used to deliver specific genes within the adult nervous system. Viral vectors can also be adapted for intracerebral delivery using implanted viral particles. Initial results indicate that neurological function can be altered by this method. Further studies are needed to determine the long-term effects of neuronal transduction on gene expression and neurological function. Additionally, the potential for viral vectors to induce inflammatory responses and the host range of specific neurological viral vectors need to be studied further.

570.8 Alterations in Levels of Choline Acetyltransferase in Somatostatin Transgenic Mice. M.L. Oster-Granite, C.F. Hohmann, S.L. Kinsman, and T.T. Coyle, Departments of Psychiatry, Physiology, and Neuroscience, The Johns Hopkins University School of Medicine, and The Kennedy Research Institute, Baltimore, Maryland.

Various studies of normal and mutant (e.g., dwarf) mice during development have indicated a dynamic interaction between the cholinergic and somatostatinergic systems in the fetal brain. Recent evidence suggests that, at a certain degree of efficiency, a complex feedback mechanism regulates cholinergic activity in the corticothalamic system. We investigated the effect of increased somatostatin levels in the brains of transgenic mice on the expression of cholinergic markers.

Using transgenesis to introduce extra copies of a 15 kb mouse genomic fragment, we investigated the effect of increased somatostatin levels in the brains of transgenic mice on the expression of cholinergic markers. Levels of choline acetyltransferase were elevated an average of 1.5 fold in neocortex, striatum, and hippocampus, but were not elevated in the remaining brain regions. Transgene expression leads to changes in the expression of cholinergic markers in the brain of transgenic mice. These data suggest that the expression of somatostatin in transgenic mice is associated with changes in cholinergic markers.
571.3 PAROXYSMAL INHIBITORY POTENTIALS MODULATED BY GABA<sub>B</sub> RECEPTORS IN DISINHIBITED HIPPOCAMPAL SLICE CULTURES. Mariano Scarnati, Beat H. Gähwiler, Margaret Schlipf, and Scott M. Thompson. Brain Research Institute, Univ. of Zurich, 8029 Zurich, Switzerland

What is the role of GABA<sub>B</sub> receptors in the regulation of paroxysmal inhibitory potentials (IPSPs) in hippocampal slice cultures?

571.4 A REVERSIBLE LOSS OF DENDRITIC SPINES ACCOMPANYING A DECREASE IN EXCITABILITY IN A MODEL OF CHRONIC LTP IN VITRO. Michael S. Vivero, Beat H. Gähwiler, Esther Petersen and Scott M. Thompson. Brain Research Institute, University of Zurich, 8029 Zurich, Switzerland.

Chronic epilepsy was induced in mature hippocampal slice cultures by adding bicuculline methochloride (BMC) or picrotoxin to the medium for 3 days. After 3 days, there was vacuolization of the soma, the numbers of spines in HRP injected cells was considerably reduced, and dendritic swellings appeared. The damage affected CA1, CA3 and granule cells. Intraacellular recordings showed a significant reduction of an EPSP synaptic activity. However, ISPs could still be evoked, and were similar to those in untreated cultures. GABA immunohistochemistry suggested that interneurons were intact in treated cultures. Acute addition of BMC to control cultures resulted in spontaneous, long-lasting (> 5 s) ieral bursts followed by spontaneous and evoked interictal spikes (< 1 s). In treated cultures, however, ictal bursts were never observed, although interictal bursts were still evoked. Moreover, Trim's staining of treated cultures suggested either a loss of mossy fiber terminals, or a depletion of zinc. BMC-treated cultures returned to normal medium for 1 week appeared to have recovered; high levels of spontaneous activity were observed, HRP injected cells possessed normal spines, and acute application of BMC resulted in spontaneous ictal bursts. The data suggest that chronic epilepiform activity causes a transient decrease in excitability, perhaps as a result of reversible morphological changes.

571.5 THE EFFECTS OF EXTRACELLULAR POTASSIUM ON EPILEPTIFORM BURST DYNAMICS IN CULTURED MONOLAYER NEURONAL NETWORKS. B.K. Rhoades and G.W. Gross. Dept. of Biological Sciences, Univ. of North Texas, Denton, TX 76203.

Neuronal cultures from embryonic mouse CNS tissue form networks whose native, spontaneous activity is characterized by the generation of spikes interspersed in a coordinated, patterned bursting. This activity may be regarded as epileptic with regards to its coarse-grained synchrony at multiple sites across the networks, its pharmacological responses to convulsant and anticonvulsant drugs, and its response to the external ionic milieu. Dissociated cell cultures of 100-200 neurons and underlying glial cells were established from the whole spinal cord, ventral spinal cord, and olfactory bulb, taken at embryonic day 14. For explant cultures, transverse spinal cord slices (400μm) were transferred to culture at the same age. The culture substrate was a multiaminoacid-based plate, with 48 phototreated electrodes forming a 16x3 extracellular recording matrix. Extracellular K+ levels were varied by KCl additions to the bathing medium. Each culture was tested in the native activity state and in a disinhibited state produced by 30mM bicuculline. In the native state, increasing extracellular potassium promoted burst consolidation and burst periodicity. Dissociation produced more periodic patterning of bursts and more uniform burst amplitudes and durations. In the disinhibited state, increasing extracellular potassium promoted burst periodicity, increased burst rate, decreased burst duration, and decreased burst amplitude. In both states increasing extracellular potassium promoted synchronization of bursts across the network. These results held for both dissociated cell and explant tissue slice cultures. The results also parallel those reported for induced epileptiform burstting in the adult hippocampal slice, and support the cultured monolayer neuronal network as an in vitro model of epileptic activity. (Supported by grants from the Texas Advanced Research Program and the Hillcrest Foundation of Dallas, founded by Mrs W.W. Caruth, Sr.)

571.6 COOPERATIVE PHENOMENA IN NEURONAL NETWORKS: DYNAMICAL MODELS OF EPILEPTIFORM ACTIVITY IN SMALL NEURONAL ENSEMBLES. L.M. Kowalski, G.L. Albert, B.K. Rhoades, and G.W. Gross. Dept. of Physics* and Biological Sciences, Univ. of North Texas, Denton, TX 76203.

A new class of neuronal network models is being developed (Kowalski & Gross, 1990) where the basic structural units are neurons of Hodgkin-Huxley type and synaptic interactions are represented by a simple model of the neurotransmitter release and subsequent generation of postsynaptic micromovements. Each neuron is governed by a scalar equation for membrane potential, a subsystem of kinetic equations for ionic variables, and equations describing neurotransmitter kinetics at synaptic junctions. The model can accommodate a wide array of interconnection schemes and types of interactions, and allows parametric changes in ionic gradients and conductances. Networks composed of non-endogenous bursters bifurcate into approximately synchronous and phase-locked bursting states. Local network disinhibition results in the emergence of activity patches which may grow, decay, or remain stationary. The model qualitatively mimics some observed effects of extracellular (K+) on epileptiform bursting in neuronal cultures from embryonic mouse CNS tissue (Rhoades & Gross, this conf.). Simulations with excitatorily interconnected networks match the experimental results from pharmacologically disinhibited cultures; in that increasing extracellular (K+) decreases both burst and interburst durations. The inclusion of inhibitory interactions may reveal the more complex (K+<sub>L</sub>) effects observed in non-disinhibited cultures. With the further elaboration of network substructure, we expect the model to also show rapid state transitions to synchronized bursting and the local emergence and network spread of coordinated activity patterns. (Funded by grants from the Texas Advanced Research Program and the Hillcrest Foundation of Dallas, founded by Mrs W.W. Caruth, Sr.)

571.7 SPONTANEOUS SYNAPTIC POTENTIALS IN CULTURED SPINAL CORD NEURONS DURING DEVELOPMENT OF EARLY CONVULSANT ACTIVITY PRODUCED BY TETANUS TOXIN. G.K. Berger and P.J. Fransenau.† Departments of Neurology and Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Tetanus toxin is distinct from other convulsants in acting at a presynaptic locus to produce a gradual onset of paroxysmal depolarizing events (PDE) following a dose dependent period (Berger et al., 1983, 1987). To investigate the changes in synaptic activity occurring during the evolution of convulsant activity, an agrin-specific detection of spontaneous postynaptic potentials (Fransenau and Berger, submitted). Intracellular recordings from dissociated mouse spinal cord neurons in culture (4-10 wk of age) following a 60 min (100:1000 ng/ml final concentration) exposure to 200-600 PPS/min were detected during the period prior to the onset of established convulsant activity. During this time period the absolute numbers of spontaneously occurring EPSPs increased while the amplitudes of the observed spontaneously occurring EPSPs remained constant or diminished. The numbers of spontaneously occurring EPSPs decreased and the amplitudes of the EPSPs increased. Spontaneously occurring large EPSPs disappeared during the period preceding organized convulsant activity. Following toxi administration, spontaneous EPSPs clustered distributed in the absence of convulsant activity (PDE).

Tetanus toxin provides a model for evaluation of convulsant action resulting from increased network excitation produced by reduced synaptic inhibition and increased excitatory activity. The possibility of synaptic pruning exists to suggest augmentation of monosynaptic excitation in this model.

571.8 IMMATURE RAT CA1 SHOWS AN INCREASED PROPENSITY FOR NON-SYNAPTIC EPILEPTIFORM ACTIVITY. S.N. Roger†, A. Osenbauer and F.E. Dudek, Mental Retardation Research Center, UCLA Division of Neurosurgery, UCLA School of Medicine, Los Angeles, CA 90024.

Immature hippocampal slices show an increased propensity for epileptiform activity in several in vitro preparations. In the absence of synaptic activity, the low-[Ca<sup>2+</sup>]<sub>E</sub> model produces synchronous population spikes that are enhanced by osmotically reducing the size of the extracellular space (Dudek et al., Neurosci. Lett. 120:267-270, 1990). Immature hippocampal slices (N=10/group) were obtained from 2- to 3-week-old rats aged 7-10 days (1 week), 11-14 days (2 weeks), 20-23 days (3 weeks), and >60 days (adult). Extracellular recordings were made from the CA1 area of the hippocampus in a low-[Ca<sup>2+</sup>]<sub>E</sub> solution, low-[Ca<sup>2+</sup>]<sub>E</sub> diluted 20% with H<sub>2</sub>O, and low-[Ca<sup>2+</sup>]<sub>E</sub> +10 mM mannitol. Slices from all age groups, except 1 week, generated bursts in the control low-[Ca<sup>2+</sup>]<sub>E</sub> solution (2 weeks 60%, 3 weeks 80%, adults 40%). In low-[Ca<sup>2+</sup>]<sub>E</sub> diluted 20% with H<sub>2</sub>O, 90%, 80%, and 40% of slices showed bursting activity in the 2-week, 3-week, and adult groups, respectively. In both of the low-[Ca<sup>2+</sup>]<sub>E</sub> solutions, burst duration was two-fold greater in the 2- and 3-week old groups compared to adults. This study suggests that CA1 hippocampal neurons from 2-3-week-old rats are more susceptible than adults to low-[Ca<sup>2+</sup>]<sub>E</sub>-induced epileptiform activity; this implies that the increased propensity of immature hippocampus for seizure activity is not dependent on synaptic mechanisms. Supported by NIH grant HD05958.
571.9
GIANT IPSC'S RECORDED IN HIPPOCAMPAL PYRAMIDAL CELLS IN THE PRESENCE OF EXCITATORY AMINO ACID BLOCKERS.
Whole-cell clamp recordings from pyramidal neurons in the CA3 region of the hippocampus in slices from guinea pig hippocampus in the presence of 4-aminopyridine (75 μM) large slow EPSCs were observed that were composed of a giant IPSC (500-2000 pA, varying among slices) outward current. The giant IPSCs were recorded in the presence of bicuculline (5 μM) and CPP (10 μM). The intracellular solution consisted of (mm) potassium glutamate 120, Hepes 10, MgATP 10, and NaCl 10. The slow IPSC's had two components. One component reversed at the same potential as the spontaneous unitary IPSC's; therefore it probably is a GABA-mediated chloride current. The other component reversed at a more negative potential. The giant IPSC's presumably reflect the synchronous firing of inhibitory interneurons. The amplitude of the giant IPSC's was 25-85 times the amplitude of the unitary IPSC's. This number can be used to estimate the number of inhibitory interneurons innervating a single pyramidal cell in a hippocampal slice.
(Supported by NIH and by a NSF Graduate Fellowship to KLP.)

571.11
HYPOXIA MODULATES THE EPILEPTIFORM ACTIVITIES INDUCED BY MAGNESIUM-FREE MEDIUM IN SLICES OF RAT HIPPOCAMPUS-ENTORHINAL CORTEX.
Combined slices of rat hippocampus (HC) and entorhinal cortex (EC) exhibit electrophysiological seizures when exposed to medium containing no added magnesium (0-Mg medium). These seizures are eventually replaced by interictal bursts (IB). It has been previously reported that the IBs, arising in CA3, disturb the seizure generation in EC. We have hypothesized that the suppression of IBs in CA3 promotes seizures in EC. Here we describe that mild hypoxia, suppressing IBs in CA3, promotes seizure generation in EC. Extracellular recordings were made in area CA3 and HC of HC/EC slices of male Sprague-Dawley rats using a submersion chamber. The bath PO2 was monitored with a Clark-type oxygen electrode in some cases (n=13). In the 0-Mg medium, seizures appeared first but eventually they gave way to IBs in both regions. After the seizures were completely replaced by IBs, mild hypoxia was introduced by switching the oxygen gas from 95%O2+5%CO2 (bath PO2=551 mmHg) to 30%O2+65%N2+5%CO2 (bath PO2=211 mmHg). In all the slices tested (n=19), the IBs were suppressed in CA3 and the seizures reappeared in EC during the hypoxic period. In regular artificial cerebrospinal fluid, orthodromic population spikes in CA3 were almost completely suppressed by the mild hypoxia whereas those in EC were less affected (n=10).
In conclusion, hippocampal area CA3 is more susceptible to hypoxia than EC and the suppression of IBs in CA3 by mild hypoxia promotes seizures in EC.

571.12
BICUCULLINE-INDUCED INTERICTAL BURSTING DECREASES THE DURATION OF STIMULUS-INDUCED ICTAL BURSTING IN THE RAT HIPPOCAMPAL SLICE.
Debbie S. Barr and Wilke A. Marson, Deps. of Pharmacology and Medicine, Duke Univ. and V.A. Medical Centers, Durham, N.C.
We demonstrated previously that in a 0-Mg2+ model of electrophysiological seizures (EGS), reduced bicuculline (10 μM) significantly decreased EGS duration in CA3 in 0-Mg2+ slices. In the present experiments, interictal (II) activity suppressed ictal activity; application of the GABAa antagonist bicuculline (24-μM) inhibited II bursting and reversed this effect. We now show similar results using status-induced EGS in the in vitro hippocampal slice. EGS's were induced in the slice preparation by repeated stimulus trains (Science, vol 245, p. 464-465, 1989), i.e., the GABAa antagonist bicuculline (10 μM) was added to the bath to initiate II bursting. EGS duration was reduced markedly in 11 of 12 experiments. Bicuculline (1 μM) was then applied to the bath in the presence of bicuculline to suppress all II activity. Application of bicuculline allowed partial to near complete recovery of EGS duration in 9 of 10 slices. In 8 of 9 experiments, interictals were simulated electrically in the presence of bicuculline and baclofen, and EGS duration was once again significantly diminished.
II bursting is viewed clinically as a symptom of epileptiform activity. These studies suggest that II bursting may actually exert negative feedback control of ictal burst duration.

571.13
GAD mRNA INCREASES IN NEURONS FROM CHRONIC EPILEPTIC FOCS INDUCED IN RATS BY INTRAHIPPOCAMPAL TETANUS TOXIN.
Intrahippocampal tetanus toxin induces chronic epileptic foci in both injected and contralateral hippocampi. Epileptic activity occurs intermittently for at least 2 weeks, usually by 2-5 weeks, and is associated with impaired GABAergic inhibition. The low dose of toxin used (ca. 10 ng) caused no gross histopathology. Instead, we used in situ hybridization (ISHH) of mRNA for GAD to determine the location and extent of increased GAD mRNA in neurons from the CA3 region of the hippocampus in slices from both hippocampi in every case. ISHH revealed an increase in GAD mRNA, which reached double the control value in hilar neurons. The low dose of toxin used (ca. 10 ng) caused no gross histopathology. Instead, we used in situ hybridization (ISHH) of mRNA for GAD to determine the location and extent of increased GAD mRNA in neurons from the CA3 region of the hippocampus in slices from both hippocampi in every case. ISHH revealed an increase in GAD mRNA, which reached double the control value in hilar neurons.

571.14
INHIBITION IN PRIMARY AND SECONDARY CHRONIC EPILEPTIC FOCI INDUCED BY INTRAHIPPOCAMPAL TETANUS TOXIN.
Intrahippocampal tetanus toxin induces chronic epileptic foci in both hippocampi, which remain epileptogenic for at least 6 weeks. We know that GABA release is depressed 2 weeks after bilateral injections of the toxin. Here we compare inhibitory function in the primary and independent, secondary, "mirror" foci. Hippocampal slices were prepared 10-16 days after injection of 12 mice L.D., into one hippocampus and used for 2 studies in vitro: 1. The Ca**+-dependent K*-evoked reticular release of H GABA was depressed for slices from the injected hippocampus, in accord with previous acute and chronic studies. 2. In contrast, it remained normal in the contralateral slices, although they generated similar epileptic activity. In control slices, both fast and slow IPSPs are reliably recorded from CA3 pyramidal cells following afferent stimulation. Both were consistently abolished within the primary focus in slices from injected hippocampus. In contrast, fast and slow IPSPs were present in a minority of CA3 cells from the focus in the contralateral hippocampus (respectively 4 and 8 of 17 cells). The secondary focus thus differs from the primary in retaining some inhibitory function, in spite of producing similar epileptic discharges.
Supported by the S.E.R.C. and Wellcome Trust
571.15 RHYTHMIC SLOW ACTIVITY AND OSMOTIC CHANGE IN CA3 PYRAMIDAL OF RAT HIPPOCAMPUS. K.P. Ogrenbo* and R.D. Andrew. Anatomy Department, Queen's University, Kingston, Ontario K7L 3N6

Osmolality inversely affects epileptiform activity both in vivo and in vitro. (Ogrenbo, J. Neurosci. 101, 7-18) Rhythmic slow activity (RSA) encodes memory in lower mammals and represents a non-pathological oscillatory cortical network. We have examined the effect of osmolality on the duration and frequency of RSA which consists of 5-30 Hz oscillations (theta rhythm) of reduced amplitude in CA3 neurons. Artificial CSF (ACSF) elevated to 40 mM with mannitol slowed SD frequency (16 of 16 cells). Conversely -40 mMACSF increased SD frequency (9 of 13). Glycerol however (+40 mOsm) had no effect (-6 of 4), indicating that the frequency effects were osmotic in origin. There was no consistent change in theta frequency (n=9) but overriding action potentials were often reduced or arrested in hyperosmotic ACSF (8 of 12). The lack of osmotic effect on theta, which is intrinsically generated by CA cells (and synchronically synchronized) fits with previous data showing no endogenous or synaptic CA3 changes in ±40 mM ACSF. On the other hand, changes in SD frequency and action potential firing may reflect elevated accommodation and slower clearing of K from the reduced extracellular space induced by hyperosmotic ACSF. Conversely, hyperosmolality may reduce [K+]out, lowering SD frequency. This hypothesis will be investigated further.

571.16 OSMOTIC EFFECTS UPON FIELD POTENTIALS: EXCITABILITY CHANGE OR SIMPLY RESISTIVE CHANGE? S.J. Quackenbush*, J. Goh and S. J. Olympic*. McGill University, Montreal, Quebec, H3A 2K6

Lowered osmolality leads to increased seizure susceptibility both in vivo and in brain slice preparations. We have shown that: 1) SD episodes can be generated in immature tissue under conditions that do not provoke them in adult tissue. Most slices were recorded in CA3, preceding or following that in CA1 area. The negative field potential accompanying SD had an amplitude of 18.1±1.1mV (mean±SEM, n=31), duration ranging from 20-250 s and it occurred every 4.5-33 min. SD was increased by 107±39% at sub-maximal stimulation (n=6). The antidromic PS increased by 43±12% and 67±16% (n=9) at max and sub-max stimulation respectively. A +50 mM increase using 3-glucose decreased control ortho PS amplitude by 43±11% at max stim and 69±24% at sub-max stim (n=6). The antidiromic PS also decreased by 28±21 and 41±6% (n=9), max and sub-max respectively. The consistently greater sub-max changes suggest that the increased field potentials in hyposmotic media result from neuronal recruitment and not simply from increased extracellular resistance presented by cell expansion. Furthermore unlike the CA1 EPPSP, the preceding CA3 afferent volley was not significantly altered in ±40 and ±50 mM ACSF nor at max and sub-max stim (n=5). With a purely resistive change, both fields should be equally affected. We propose that axons and their glia are less susceptible to volume changes than somatic and dendritic regions.

571.17 EPILEPTIFORM DISCHARGES INDUCED BY TETRAETHYLAMMONIUM (TEA) IN THE RAT HIPPOCAMPAL SLICE. Y. Fueli, D.A. Apkoff, MNI and McGill Univ., Montreal, Quebec, H3A 2B4

TEA is a K+ channel blocker and a convulsant drug in the CNS. We have shown that 4-aminoypyridine (4-AP), which blocks a different K+ channel, induces epileptiform discharges and synchronous GABA-mediated depolarizing potentials in the CA3 subfield of the adult rat hippocampal slice (Andrew, R.D. J. Neurosci. 65,771,9191). Therefore intra- and extracellular recordings were performed to study whether TEA could disclose similar patterns in the CA3 subfield. Like 4-AP, TEA induced interictal field potentials, which were associated with an intracellular burst of action potentials, were blocked by the non-NMDA receptor antagonist CNQX in a dose-dependent manner, and were resistant to CPP, which is a non-NMDA antagonist. However TEA did not disclose any GABA-mediated depolarization, alternating mechanisms appeared to be operant. Our data show that TEA induces epileptiform discharges mediated through non-NMDA receptors, but it is not capable of modifying GABA-mediated transmission in a way similar to that reported for 4-AP.

Beta amyloid alters neuronal viability in dissociated hippocampal cultures, and may play a direct role in the etiology of Alzheimer's Disease (Whinston et al., Science 243, 1448-1450). We are analyzing the effects of B1-28 and B25-35 on rat hippocampal slice cultures; a culture system that maintains the organotypic structure and synaptic connectivity of the intact hippocampus. Slice cultures were exposed to 30µM B1-28 for 2-14 days, and individual CA3 pyramidal neurons were filled with Lucifer yellow or neurobiotin. No gross differences in the overall health of the cultures or morphologies of individual cells were observed between B1-28 treated and control cultures. Cumulative length and number of branch points of apical dendrites were quantified using the Eutectics image analyzer. A small increase in number of branch points was observed. Slice cultures were exposed to 50ng/ml NGF = B1-28 (30µM or 100µM), since NGF was reported to enhance the potency of beta amyloid (Yankner et al., Proc Nat Acad Sci 87, 9020-9023). Small increases in length and number of branches of the apical dendrites were observed after exposure to 30µM B1-28 + NGF. We are presently examining the effects of B25-35 (100µM) on hippocampal slice cultures. B25-35 has been reported to be more potent than B1-28 (Yankner et al., Science 250, 279-282). Cultures exposed to B25-35 for 24 hr appear less healthy than untreated controls. Cumulative dendritic length and number of branch points are being quantified. Beta amyloid appears to have small effects on the viability of neurons in hippocampal slice cultures.

Supported by Alzheimer's Assoc. Grant 1R01-90-126.


We wish to understand how and why BAP levels increase in AD brain. Using an SN1 suicide protection assay, total APP RNA levels increase 2 to 3 fold (p<0.01) in 16 vs. 10 normal brains (Jabbenstein, et al. Neurobiol. Aging 12[1991] in press) which partially explains the increased BAP levels in AD. The remainder may be due to protein processing of the Amyloid Peptide Precursor (APP) into BAP. To test this, we have reconstructed a marked APP substrate with Beta-endoPeptidase (BAPase) enzymes. APP must be cut at the ends of its internal BAP domain by amino-BAPase (N-BAPase) and carboxy-BAPase (C-BAPase) activities which may change in AD. Alternatively, the Dutch and London mutations found in the BAP region of APP are designed by the rate of APP degradations. In addition, we have engineered analogs of APP to contain RPEPoter peptides flanking the BAP domain. APP precursor cleavage of APPRE is followed by immunological detection of reporter associated with protein fragments whose lengths reflect cleavage location. We have expressed and specifically detected APPRE with antibodies to the Kunht Protease inhibitor domain of APP and to Substance-P. Further characterization of APPRE with additional antibodies, agonistic sequencing, peptide mapping and HPLC analysis are in progress.


Although β/α4-protein was found to be a major constituent of amyloid in Alzheimer's disease (AD) brain, other components might also be found in AD amyloid. Recently, we have discovered a new component of amyloid from AD brain and cloned the corresponding cDNA. RNA homologous sequence is found to be repeated in it. The DNA sequence demonstrated a striking feature of NAC, i.e., five K#K##V sequences in this 82-amino acid stretch. Furthermore, a 10-amino acid homologous sequence is found to be repeated in it. The characterization of NAC should shed light on the amyloidogenesis in AD and may help in the search for the etiology of this disease.


Using antibodies against full length β/α4 protein, we have synthesized and secretion of AβP in human brain neuroglial cells (ATCC HIB 148) by using reverse transcriptase polymerase chain reaction. The cDNA was cloned in the presence of SDS, solubilized in formic acid, digested with cyanogen bromide, and then deglycosylated and analyzed by HPLC. Analysis of the amino acid sequence showed the following in addition to the β/α4 peptide fragment, a peptide with unknown sequence. We named this peptide NAC signifying New Amyloid Component. This sequence was used to raise a rabbit antiserum, which stained the amyloid core of neuritic plaques on brain sections and a 40kD protein in Western blots. Oligonucleotides were generated from this sequence to amplify the cDNA from human brain cDNA library using PCR. A 247-nucleotide DNA was amplified. DNA sequencing showed the PCR product to contain a nucleotide stretch which encodes the exact sequence of NAC. The amino acid sequence deduced from the DNA sequence demonstrated a striking feature of NAC, i.e., five K#K##V sequences in this 82-amino acid stretch. Furthermore, a 10-amino acid homologous sequence is found to be repeated in it. The characterization of NAC should shed light on the amyloidogenesis in AD and may help in the search for the etiology of this disease.
572.7 Participation of the Amyloid A4 Precursor Protein in Lymphocyte Activation
Yoo-Ran Shuh, Woon Choi*, Jee-Bun Kim*, Sung-Soo Kim*, Joon-In NooP and Chan Wook Park*
Dept. of Pharmacology, Seoul National University College of Medicine Dept. of Molecular Biology, Neuroscience Research Institute, Seoul National University, Seoul 110-460, Korea

Amyloid deposition in senile plaques and the cerebral vasculature is a marker of Alzheimer disease. It is encoded as part of a larger precursor (Pre A4) that maps to chromosome 21. Three mRNA-bands have now been accounted for by the demonstration of three alternative splicing products.

The precursor proteins (Pre A4.9, Pre A4.9.5, and Pre A4.5.9) of Alzheimer disease A4 amyloid protein are integral, N-glycosylated membrane proteins of unknown function.

Here we report that normal human lymphocytes express pre A4 mRNA and protein. Pre A4 is detectable on the surface of lymphocytes. Lymphocyte Pre A4 surface abundance is increased by cell activation. We conclude that Pre A4 is a lymphocyte surface molecule that may participate in cell activation.

572.8 Phagocytosis and Vascular Deposition of Alzheimer Amyloid in Rat. S.A. Frautschy, G.M. Cole and A. Baird, Dept. of Cellular and Molecular Biology, Whittier Institute, 9894 Genesis, La Jolla, Ca 92037 and Dept. of Neurosciences, UCSD, La Jolla, Ca. 92037

Deposits of extracellular β amyloid occur in plaques and vessels in Alzheimer's disease(AD), but they are not found in normal rodent aging, nor have they been induced. In order to develop an animal model for β amyloidosis, we injected amyloid cores from AD brains and, contralaterally, similarly lipofuscin fractions into cortex and hippocampus of adult rats. Rats were sacrificed 2 d (n=2), 7 d (n=2) and 30d (n=10) and 60d (n=2) after injection and sections were silver or Congo red stained or immunostained for β amyloid. Amyloid and lipofuscin moved medially and laterally from the injection site and persisted for at least 2 months. The astroglial and microglial responses indexed by glial fibrillary acidic protein and OX-42 immunostaining were similar on the amyloid and control sides. By 7d much of the amyloid was in or near phagocytes which appeared to migrate to the vessels and the ventricles. At 30d, there was clear vascular β protein immunostaining. These results show that in the rat, AD amyloid deposits in the neocopl can be aggressively phagocytosed and deposited in vessels.

This work was supported by NIH grants DK18811 (A.B.), NS28121 (A.B.), AG09009 (G.M.C.) and a contract from the Calif. Dept. Health Services (G.M.C.).

572.9 MULTIPLE CLEAVAGE SITES OF ALZHEIMER AMYLOID PRECURSOR IN NEURONALLY DIFFERENTIATED PC-12 CELLS J. Anderson, F. Esch, P. Rem, K. Sambamurti, R. Mathews and R. Rehakis
Dept. of Psychiatry, Mt. Sinai Med Ctr, New York, NY 10029 and Athena Neurosciences, S. San Francisco, CA 94080

Studies in transfected cells have shown that the secreted form of Alzheimer amyloid precursor protein (APP) is cleaved by APP695

Here we report that normal human lymphocytes express pre A4 mRNA and protein. Pre A4 is detectable on the surface of lymphocytes. Lymphocyte Pre A4 surface abundance is increased by cell activation. We conclude that Pre A4 is a lymphocyte surface molecule that may participate in cell activation.

572.10 DISTRIBUTION AND ULTRASTRUCTURE OF CEREBROVASCULAR AMYLOID IN AGED SQUIRREL MONKEYS. L.C. Walker and D.L. Price, Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cerebrovascular amyloidosis occurs in the context of several neurological disorders, including Alzheimer's disease. Aged squirrel monkeys (Saimiri sciureus) often develop cerebrovascular amyloidosis that is similar in distribution to that of humans. In immunocytochemical studies of animals 18-23 years of age, we have found that: vascular amyloid deposition can be pronounced in the late teens; the incidence and distribution of vascular (and nonvascular) amyloid vary markedly among animals of similar age; and the focal clustering of lesions, which is particularly apparent in mildly affected brain areas, suggests selective involvement of the branches of a common blood vessel. Ultrastructurally, various vascular abnormalities are evident, and foamy, debris- and lipid-laden macrophages often about amyloidotic vessels. These findings indicate that cerebral amyloid angiopathy in aged squirrel monkeys can selectively affect certain vessels, that the pattern of lesions varies among individuals, and that macrophages may play a role in the processing of mural amyloid in cerebral blood vessels.

572.11 STRESS-INDUCED ALTERATION OF APP mRNAs. B. Border, S. Pardus*, E.K. Miller* and M. Morrison-Bogorad. Dept. of Neurology, Southwestern Medical School, Dallas TX 75235

Although amyloid deposition may be related to changes in absolute or relative amounts of different amyloid precursor proteins (APPs), factors regulating their levels in vivo are unknown. We have shown that two in vivo stressors result in rapid changes in specific APP mRNA levels in rat hippocampus. Rats were made hyperthermic by exposure to heat or injection of amphetamine and sacrificed 2 hours after attaining a temperature of 42°C. Hybridization to an 18s rRNA oligonucleotide probe revealed similar levels of hybridization to neurons in the dentate gyrus and CA1-4 regions in all rats, indicating that signal intensity with mRNA-specific probes could be directly compared. Heat shock alone did not change overall levels of the APP mRNAs, whereas they were increased after amphetamine-induced hyperthermia. There was a reduction in levels of the APP695 mRNA in heat shocked rats but a strong induction in the amphetamine-treated rats. Our results suggest that there is a several fold change in the relative amounts of different APP mRNAs after heat shock, while amphetamine induction of both total APP and APP695 mRNAs show a separate drug effect over and above that produced by heat shock. Supported by NIH AG08013


While the amyloid beta (1-40) sequence of the amyloid precursor protein (APP) is commonly cited as the source of the extracellular β amyloid, other peptides have been shown to be present in senile plaques. Using a monoclonal antibody directed against a specific APP sequence ( designed to label the Kunitz inhibitor domain). We have examined the distribution and co-localization of APP immunostaining using a monoclonal APP antibody and a polyclonal antibody against β/4. In some AD cases showed the strongest staining within the hilus while other cases had no staining. Total plaque number varied across regions examined, but anti-APP, anti-β/4 or Bielskowsky staining did not show any significant number of plaques within each region except parahippocampal cortex, where the number of β/4 positive plaques was more than three times greater than APP positive plaques. APP positive plaques were composed of aggregates of punctate, granular staining and were positive in plaques within the end terminal region of the amyloid precursor protein (APP) containing the Kunitz inhibitor domain. We have examined the distribution and co-localization of APP immunostaining using a monoclonal APP antibody and a polyclonal antibody against β/4. We found that this APP antibody is sensitive to fixation and yields the best results in embalmed tissue. No APP positive staining was observed in controls. Within AD brains, the distribution of APP positive plaques varied greatly, e.g. some AD cases showed the strongest staining within the hilus while other cases had no apparent staining. Total plaque number varied across regions examined, but anti-APP, anti-β/4 or Bielskowsky staining did not show any significant number of plaques within each region except parahippocampal cortex, where the number of β/4 positive plaques was more than three times greater than APP positive plaques. APP positive plaques were composed of aggregates of punctate, granular staining and were co-localized with thioflavin S. APP positive staining was not limited to plaque-like structures: pyramidal neurons of fields CA3 through CA1, curvy fibers surrounding and entering plaques and some blood vessels were also stained. Using confocal microscopy and double labeling, we found that all APP positive plaques were also positive for β/4, and many β/4 positive plaques did not contain APP. These data are consistent with the possibility that APP is present in immature plaques and is degraded to form β/4 plaques.
572.13

β-AMYLOID PROTEIN POTENTIALITY INJURED BY GLUCOSE DEPRIVATION IN NEURONAL CORTICAL CULTURES, A.G. Copand, J. Parada & C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717 U.S.A.

Alzheimer's disease (AD) is associated with a generalized decline in cerebral metabolism, which occurs most severely in the temporal and parietal cortex. In the present study, we used an in vitro paradigm to examine the possibility that β-amyloid protein deposition in AD plaques may increase neuronal vulnerability to glucose deprivation. Mature murine cortical cultures deprived of glucose for 3-6 hours developed neuronal degeneration estimated both by examination with phase-contrast microscopy and by lactate dehydrogenase assay. This toxicity was increased when cultures were simultaneously exposed to 0.1-100 μg/ml synthetic β-amyloid (1-42). Cultures treated with β1-42 were reported to induce glutamate neurotoxicity (L. Koh et al., 1990); thus the increase in hypoglycemic neurotoxicity by β1-42 may be due to the potentiation of endogenous excitatory amino acid neurotransmitters which have been reported to activate glucose deprivation injury. Consistent with this idea, the antagonist of NMDA receptor MK801 (5μM) largely attenuated the toxicity-increasing effect of β1-42. The present results suggest that β-amyloid protein could exacerbate the neural cell loss in the presence of a defective cerebral energy metabolism occurring in Alzheimer's disease.

572.15

SITE-SPECIFIC KICKING OF ALZHEIMER B/A4 AMYLOID ALTERS THE AGGREGATION STATE. T. Honda and C.A. Nicotera. MGH, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

β/A4 Amyloid peptide (1-28) aggregated to 150kDa on SDS/acrylamide PAGE. A mix of peptides readily degrades the complex, endoproteinase Arg-C produced a new aggregate of higher molecular weight (160kDa) without denaturing gels without degraded smaller products. The new aggregate was composed of three peptides: β/A4 (1-28), β/A4 (1-5) and β/A4 (6-28). The same results were obtained by treatment of β/A4 with other arginine-specific proteases: the γ-secretase Nerve Growth Factor and clonotatin. The results indicate that arginine-specific proteases, including a growth factor processing enzyme, can nick aggregated β/A4 amyloid and alter the configuration to produce a more complex form. Partial cleavagings, or nicking, of this type occur in the AD brain then proteolytic processes themselves may promote aggregation of amyloid. This mechanism may contribute to formation of the relatively insoluble amyloid core of senile plaques. Supported by grants from Mitsubishi Kasei Corporation, the National Institute on Aging (AG0124576) and a Metropolitan Life Foundation Award to C.A.M.

572.16

HUMAN MICROGLIA EXPRESS AMYLOID PRECURSOR PROTEIN. D.M. Dickens, S. Lee, L.A. Mattice, N. Liu, A. Fishman, J.J. Lipton. Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461.

Microglia are associated with virtually every amyloid deposit in Alzheimer brains. Amyloid is composed of a 4kD molecule (β/A4) derived from a larger precursor protein (APP) with several alternative transcripts, including one that is identical to protease nexin-2. APP is wide more in rodent brain, lacks a trated in a number of tissues, and it has trophic effects on neuronal cells. In order to study proline nest in feral brains, no significant difference in the APP mRNA content of the CA1, or CA3 layer. No change in the prevalence of APP-695 mRNA was detected.

572.17

SYNTHETIC β-AMYLOID IS NEUROTOXIC IN VITRO FOLLOWING AGING OF PEPTIDE. C.J. Pike, A.L. Waksenezic, C.G. Glabe and C.W. Cotman. Departments of Psychology and Molecular Biology and Biochemistry, University of California, Irvine, CA 92717 USA.

Beta-amyloid is an approximately 42 amino acid residue protein and is the major component of the senile plaques found in Alzheimer's disease. We have found that short-term incubation of synthetic β-1-42 solution can result in the formation of peptide aggregations. In order to characterize the biological activity of these β-1-42 aggregates, we have conducted experiments comparing the effects of freshly solubilized and pre-incubated β1-42 on neuronal survival. Amyloid peptide was added to immature hippocampal cultures (16,000 cells/cm2) from embryonic (E-18) rats at concentrations of 0.1, 1, 10, 100, and 1000 μg/ml. After twenty-four hours under these conditions, cultures treated with freshly solubilized β1-42 showed increased neurite-outgrowth at both the 50 μg/ml and 100 μg/ml concentrations. The use of pre-incubated β1-42 had more than the 100 μg/ml concentration. Both the neurite-enhancement and neurotoxicity effects were dose-dependent, showing differences from controls only above the 10 μg/ml concentration. Analysis of SDS-PAGE revealed that freshly solubilized β1-42 migrated primarily as a 4-kD monomer whereas pre-incubated β1-42 had additional high molecular weight bands. These data suggest that pre-incubation of β1-42 results in both a change in biological activity from peptide-promoting to neurotoxic and the formation of SDS-resistant peptide aggregates. These findings are consistent with the hypothesis that neurodegeneration in Alzheimer's disease is related to insoluble β-amyloid found in senile plaques.

572.18


Results were obtained by treatment of β/A4 with other arginine-specific proteases: the γ-secretase Nerve Growth Factor and clonotatin. The results indicate that arginine-specific proteases, including a growth factor processing enzyme, can nick aggregated β/A4 amyloid and alter the configuration to produce a more complex form. Partial cleavagings, or nicking, of this type occur in the AD brain then proteolytic processes themselves may promote aggregation of amyloid. This mechanism may contribute to formation of the relatively insoluble amyloid core of senile plaques. Supported by grants from Mitsubishi Kasei Corporation, the National Institute on Aging (AG0124576) and a Metropolitan Life Foundation Award to C.A.M.

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572.20

SYNTHETIC β-AMYLOID IS NEUROTOXIC IN VITRO FOLLOWING AGING OF PEPTIDE. C.J. Pike, A.L. Waksenezic, C.G. Glabe and C.W. Cotman. Departments of Psychology and Molecular Biology and Biochemistry, University of California, Irvine, CA 92717 USA.

Beta-amyloid is an approximately 42 amino acid residue protein and is the major component of the senile plaques found in Alzheimer's disease. We have found that short-term incubation of synthetic β-1-42 solution can result in the formation of peptide aggregations. In order to characterize the biological activity of these β-1-42 aggregates, we have conducted experiments comparing the effects of freshly solubilized and pre-incubated β1-42 on neuronal survival. Amyloid peptide was added to immature hippocampal cultures (16,000 cells/cm2) from embryonic (E-18) rats at concentrations of 0.1, 1, 10, 100, and 1000 μg/ml. After twenty-four hours under these conditions, cultures treated with freshly solubilized β1-42 showed increased neurite-outgrowth at both the 50 μg/ml and 100 μg/ml concentrations. The use of pre-incubated β1-42 had more than the 100 μg/ml concentration. Both the neurite-enhancement and neurotoxicity effects were dose-dependent, showing differences from controls only above the 10 μg/ml concentration. Analysis of SDS-PAGE revealed that freshly solubilized β1-42 migrated primarily as a 4-kD monomer whereas pre-incubated β1-42 had additional high molecular weight bands. These data suggest that pre-incubation of β1-42 results in both a change in biological activity from peptide-promoting to neurotoxic and the formation of SDS-resistant peptide aggregates. These findings are consistent with the hypothesis that neurodegeneration in Alzheimer's disease is related to insoluble β-amyloid found in senile plaques.
4I. Lieberburg, J.C. Clemens and D. Selkoe. Brigham & Women's Hosp., Boston, changes were found with control peptide. Experiments are currently underway to
and cortical blocks were excised and post-fixed for 4 hours. Tissue was paraffin-
weeks or 3-4 months. Monkeys were perfused with periodate-lysine-paraformaldehyde
were at least duplicate, and the animals were allowed to survive for 2-3 weeks, 8-10
BAP 25-35; or SDS-insoluble particles purified from control cortex. All injections
artificial CSF or 35% acetonitrile/0.1% TFA (AN); control samples were identically

trophic effects of βAP in vitro. Since βAP deposition and surrounding neuritic
that are closely reminiscent of AD pathology have not yet been seen.

that are closely reminiscent of AD pathology have not yet been seen.

LIEBERBURG, J.C. CLEMENS and D. SELKOE. BRIGHAM & WOMEN'S HOSP., BOSTON,

The progressive deposition of β-amyloid protein (βAP) is an early pathogenetic
studies of these have been confined to in vitro model systems, we chose to study its effects in vivo in a species that exhibits age-related βAP deposition to evaluate the effects of βAP when it is implanted into the brain in a lipophilic, non-toxic vector. Adult rhesus monkeys were anesthetized and their heads placed into a stereotactic instrument. Sterile implants of synthetic BAP 1-43 prepared in cocoa butter were made stereotactically through a stainless steel canula into the frontal cortex. The coronal hemisphere received implants of a control peptide (N-acetyl scrambled BAP 25-35) or vehicle alone. After 30 days, monkeys were perfused transcardially with periodate-lysine-parafomaldehyde. Coronal blocks were excised, processed and stained by conventional histological and immunostaining techniques. Evaluation of three monkeys to date has not revealed characteristic AD-like pathology at any of the implant sites. However, the following differences between control and experimental implant sites were observed in these initial experiments: 1) BAP implants produced larger necrotic lesions than those produced by control implants. 2) Axonal spheroids, morphologically abnormal processes and swollen neurites, as revealed by Bielschowsky silver staining, appeared to be more numerous in the BAP than the control implants. 3) BAP implants demonstrated more extensive astroglia, as observed by immunolocalization of glial fibrillary acidic protein and S-100 protein. Studies in progress are aimed at implanting more animals with varying amounts of the peptide, using other control peptides and evaluating the lesions with multisite antigens to further define the effects of BAP in the primate brain.

The progressive deposition of β-amyloid protein (βAP) is an early pathogenetic
event in Alzheimer's disease (AD). Recent studies suggest both neurotrophic and neurotrop-}

573.3 AMYLOID PRECURSOR PROTEIN (APP) IN ALZHEIMER'S DISEASE (AD): A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY. M. Gearing, E.R. Unger*, E.R. Shelton*, W.J. Chert* and S.S. Murr. VA Medical Center and Emory University School of Medicine, Atlanta, GA 30322 and Synex Research, Palo Alto, CA 94303.

Differential amyloid protein precursor (APP) gene expression was investigated in neonatal rat brain cortex, meninges, and primary astroglial, neuronal and microglial cultures. Relative to brain cortex levels, total APP mRNA was higher in meninges (4.5x) and neuronal cultures (1.4x) and lower in astroglial and microglial cell cultures (0.25x). Ribonuclease protection assays revealed that APP$_{695}$ mRNA was predominant in cortex and cortical neuron (~95% of total APP mRNA) while APP$_{573}$ containing forms were more abundant in meninges, astrocytes and microglia (~70% of total APP mRNA). The ratio of APP mRNA species changed in neurons and astrocytes with time in culture. Northern blot analysis revealed that cortical astrocytes express an additional 2.7kb APP mRNA (4) abnormal processing or high turnover of APP C-terminal fragments might occur in primary cell cultures (5) neurons and astrocytes maintained in culture undergo changes in alternative splicing of APP mRNA. Supported by NIA MA08155.


The amyloid precursor protein (APP) gene produces several transcripts. These include APP-695, that is largely neuron-specific, as well as a family of mRNAs with a Kunitz serine protease inhibitor (KPI-APP) motif which are expressed in many cell types. We have previously shown that KPI-APP transcripts are elevated relative to APP-695 in the CNS of aged rats with cognitive impairments. In the present study, the developing rat CNS was shown to contain the highest amounts of APP-695 mRNA relative to KPI-containing APP transcripts at postnatal day 15, paralleling the temporal expression of the low affinity NSF-F in the basal forebrain. Chronic infusion of human recombinant NGF into the striatum of adult rats resulted in an induction of APP-695 mRNA, reiterating the pattern of APP expression observed in the developing basal forebrain. These results demonstrate that trophic factors such as NGF can regulate APP mRNA splicing in oto.


The expression of the C-terminal 100 (APP-C100) residues of the amyloid precursor protein (APP) may provide a mechanism for processing of APP into the 42-43 residue beta amyloid peptide (beta 4A4) implicated in Alzheimer's disease. Expression of human APP-C100 in mammalian cells reportedly causes "toxicity" and amyloid fibrils. We have expressed the APP-C100 in human embryonic kidney cells (293 cells) in a transient assay and compared it to the expression of transfected full length APP. Products were characterized by Western blot analysis using antibody to the carboxy-terminal region of APP-C100 and the amino-terminal region of beta 4A4. The APP-C100 produced in 293 cells is the same size as the fragment left in cells after protease nexin II cleavage from full length APP (Esch et al., Science 248:1122, 1990), and is only recognized by anti-carboxy terminal antibody and therefore may not contain the amino terminal end of beta 4A4. Moreover, APP-C100 appears to be a substrate for "APP secretase" whether it is expressed with the natural APP signal peptide or as APP-C100.

573.10 Beta-AMYLOID PROTEIN POTENTIATES H$_2$O$_2$-INDUCED NEURON DEGENERATION IN VITRO. B.D. Saunders, C.A. Luttman*, P.T. Keib* and S.P. Little*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

In Alzheimer's disease, the accumulation of neuritic plaques and tangles has been associated with selective neuronal degeneration. The fact the beta-amyloid protein accumulates in these plaques suggests that it may be involved in neuronal degeneration, either by exerting a direct neurotoxicity on neurons or by making neurons more vulnerable to endogenous toxins. Free radicals and oxidants are believed to contribute to neuronal damage in various disease states. We tested the ability of beta-amyloid protein (beta 43) to increase the vulnerability of cultured hippocampal neurons to H$_2$O$_2$-induced degeneration. Neurons were incubated with beta 43 for 1 to 3 days before addition of H$_2$O$_2$. Neurons were incubated with 1, 10, 50, and 100 uM H$_2$O$_2$ for 15 min and then examined the next day for evidence of degeneration. In cultures that had not been preincubated with beta 43, only 50 and 100 uM H$_2$O$_2$ induced significant neuronal degeneration. Preincubation with beta 43 increased the susceptibility of the neurons to damage at 1 and 10 uM H$_2$O$_2$. The results suggest that beta-amyloid potentiates the toxicity that oxidants exert on neurons and therefore may increase the vulnerability of neurons to damage mediated by various endogenous toxins.


In Alzheimer's disease, beta-amyloid protein accumulates in neuritic plaques that are associated with neuronal degeneration. The fact that beta-amyloid protein accumulates in these plaques suggests that it may play a role in neuronal degeneration, either by exerting a direct neurotoxicity on neurons or by making neurons more vulnerable to endogenous toxins. We have previously shown that KPI-APP transcripts are elevated relative to APP-695 in the CNS of aged rats with cognitive impairments. In the present study, the developin rat CNS was shown to contain the highest amounts of APP-695 mRNA relative to KPI-containing APP transcripts at postnatal day 15, paralleling the temporal expression of the low affinity NSF-R in the basal forebrain. Chronic infusion of human recombinant NGF into the striatum of adult rats resulted in an induction of APP-695 mRNA, reiterating the pattern of APP expression observed in the developing basal forebrain. These results demonstrate that trophic factors such as NGF can regulate APP mRNA splicing in oto.


Recent studies have implicated carboxyterminal fragments of the Alzheimer precursor protein (APP) in the processes of amyloidogenesis and neurodegeneration. In particular, the carboxyterminal 104 amino acids of APP (termed APP-C104) exhibits neurotoxic activity in vitro and in vivo. We hypothesized that APP-C104 may cause the degeneration of neurons by interacting with a cell surface receptor. In support of this hypothesis, we have shown that APP-C104 synthesized in vitro binds specifically to and with high affinity to the surface of NGF-treated PC12 cells. Both the cell surface binding and the neurotoxicity of APP-C104 are pH dependent with peak activity at pH 7.0-7.2. Neurons exposed to APP-C104 are selectively inhibited by tachykinins. Mutational analysis suggests that both the binding and the neurotoxicity are dependent at least in part on the presence of a tyrosine residue that is a potential site of phosphorylation at the carboxyterminus of the fragment.
LACTOFERRIN IMMUNOCYTOCHEMISTRY IN ALZHEIMER BRAIN TISSUE. T. Kawanata*, B.C. Sung and P.L. McGeer. Kaminen Laboratory for Neurobiology of British Columbia, Vancouver, B.C., V6E 1W5, Canada. Lactoferrin (LF) is a protein of unknown function which occurs in many secretory fluids and in the brain. It also occurs in Alzheimer Disease (AD) plaques and tangles (Osmand and Switzer, Neurobiol Aging 11:284, 1990). We studied its distribution in brain immunohistochemically, and confirmed the report of Osmand and Switzer. However, we also found it to be present in many neurons of elderly brain, as well as in some astrocytes and microglia, but not in baby brain. At the EM level, LF immunoreactivity (IR) occurred primarily in lysosome-like particles, and was frequently associated with lipofuscin. It was also occasionally distributed diffusely in the cytoplasm. In AD, LF-IR was seen with that of Aβ-50, ubiquitin, and Tau-2 to assess its association with neurofibrillary tangles and neurofibrillary plaques. By contrast, the BAP antibody, which stains intramicroglial material, BAP deposits in microglial cells were seen communicating with neurons, suggesting that APP is concentrated in an intraneuronal organelle and is not a cell surface protein. In Alzheimer disease (AD), R36 and R37 stained the cytoplasm of some dystrophic neurites but did not stain their surface membranes and only sparsely stained extracellular deposits. By contrast, the BAP antibody (R27) did not stain intraneuronal material, indicating inaccessibility of the epitope, but did strongly stain extracellular deposits. All 3 antibodies prominently stained amyloid, and no β-amyloid protein has been detected by immunohistochemistry are in progress. Microglia process amyloid precursor protein segments in Alzheimer’s disease. E. R. Johnson*, F. P. Little*, C. A. Allsop*, T. Kawanata*, R. H. Attems and T. L. Littenberg, Kaminen Laboratory, University of British Columbia, Vancouver, B.C., Canada. The immunohistochemical distribution of APP was studied in normal and Alzheimer Disease (AD) brain with antibodies to residues 527-540 (R36), 597-620 (R17, 1-24 of BAP) and 681-695 (R37) (Beyreuther et al., J Biol Chem 264:133-147, 1989) at light and EM levels. Antibodies to segments flanking BAP (R36 and R37) prominently stained dense membrane-bound granules in normal and AD pyramidal neurons, suggesting that APP is concentrated in an intraneuronal organelle and is not a cell surface protein. In Alzheimer disease (AD), R36 and R37 stained the cytoplasm of some dystrophic neurites but did not stain their surface membranes and only sparsely stained extracellular deposits. By contrast, the BAP antibody (R27) did not stain intraneuronal material, indicating inaccessibility of the epitope, but did strongly stain extracellular deposits. 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573.19

POSITIVE UBQIQUITIN IMMUNOREACTIVITY IN CEREBRAL AMYLOID ANGIOPATHY. F.F. Cruz-Sanchez*, C. Mann, L. Ferrer* and E. Tolosa, Neurological Tissue Bank, Service of Neurology, HCP and Dept. of Pathology, HPE, Universitat de Barcelona, SPAIN.

Vascular amyloid deposition, senile plaques (SP) and neurofibrillary tangles (NFT), are encountered in both cerebral amyloid angiopathy (CAA) and Alzheimer’s disease (AD). Ubiquitin (UBQ) is a polypeptide related to ATP-dependent abnormal protein metabolism. Immunohistological studies using antibodies for UBQ have demonstrated similar antigenic determinants between (SP) in CAA and AD. In the present study, we describe immunohistochemical findings in the cerebral blood vessels of CAA compared to the AD ones. Formalin fixed paraffin embedded brain tissue from 4 AD and 4 cerebrovascular patients and from 2 elderly patients without neurological disorders were studied. Sections were stained for the polyclonal antibody for UBQ. Wall vessels in CAA cases showed strong UBQ-reaction positivity in the subendothelial region. SP showed strong positivity with a negative central core immunoreactivity. AD brains showed immunoreactivity for UBQ in SP and NFT, but, vessels were negative. Control brains showed few positive SP and NFT and vessels were negative. This results may indicate that vascular amyloid deposition in CAA and AD might have different pathophysiological mechanisms.

DEGENERATIVE DISEASE

574.1


Severe atrophy of the neostriatum is a main characteristic of Huntington’s disease (HD). Intrastriatal lesions with excitatory amino acids (EAA) mimicking the features of HD leading to the hypothesis that EAA’s play an etiologic role in HD. However, the EAA hypothesis of HD does not readily account for the striking involvement of the striatum with relative sparing of other structures which also participate in EAA circuits.

6-HydroxyDOPA (6OH-DOPA), a derivative of the dopamine precursor L-DOPA, has been reported to have neurotoxic properties (Cleary et al., Exp. Neurol. 108:269, 1990). 6OH-DOPA displaced rat [3H]AMPA binding in a autoradiographic assay. Ineffective displacers of [3H]AMPA binding included: dopamine, L-DOPA, DOPAC, homovanillic acid (HVA) and [3H]-spiroperidol (D2 receptor) binding. The Kd of 6OH-DOPA alone was determined for [3H]AMPA binding was 31.7 µM, as potent as kainate in displacing [3H]AMPA binding.

These data raise the possibility that 6OH-DOPA, or another abnormal L-DOPA metabolite, could act as an excitotoxic agent via action at AMPA receptors. If the genetic defect in HD resulted in production of 6OH-DOPA, neuronal death would be expected to occur in those structures with a coincidence of L-DOPA (as toxin precursor) and postsynaptic AMPA receptors (mediators of 6OH-DOPA-induced cell death), such as the striatum.

Supported by NIH NRSA F32 GM-07863 and USPHS Grant NS 15613.

574.2

NEUROPSYCHOLOGICAL CHANGES ASSOCIATED WITH CT SCAN MEASURES OF NEUROANATOMICAL ATROPHY IN HUNTINGTON’S DISEASE. D.A. Roberts, E.A. Loh, S.L. Sally & P. Mohs*, Dept. of Neuropsychiatry, Royal Ottawa Hospital, Ottawa, Canada, K1Z 7K4. CT scan measures of basal ganglia regions of 15 patients at various stages of the progression of Huntington’s disease (HD) were compared with patient’s scores on a neuropsychological test battery. Indices of caudate atrophy: Frontal horn of ventricle width and Bicaudate distance were determined from measures taken from digitized CT scans. Lentiform region atrophy was assessed by measures of width of the third ventricle and distance between the third ventricle and insular regions. Cognitive tests were often as well correlated with atrophy of the lentiform regions as more traditionally used measures of caudate atrophy. Of particular interest, measures of attention and perseveration were highly correlated (P<.05) with the lentiform measures even after the effects of the choreiform movements were factored out. These data suggest that measurements of the lentiform regions provide additional information on the progress of HD.

574.3

A QUANTITATIVE LAMINAR ANALYSIS OF CEREBRAL CORtical D EGENERATION IN HUNTINGTON’S DISEASE. SM. Hersch, V Hopfenthal*, C.A. Quickhust, RD. Rhee, RC Green, Department of Neurology, Emory University School of Medicine, Atlanta, Georgia 30322.

The extent and significance of cerebral cortical atrophy occurring in Huntington’s disease (HD) and its implications for understanding the symptoms and pathophysiology of the disease are of great interest. One-micron thick plastic sections, stained with toluidine blue, from defined regions of cerebral cortex from the brains of patients with Huntington’s disease (HD) are being examined using a Macintosh computer based image analysis system for data acquisition and analysis. Right hemispheres from 10 patients, aged 45-69, with grade 3 or 4 HD were obtained from the Brain Tissue Acquisition and Analysis System at the Mayo Clinic. Age-matched control tissue was obtained from routine autopsies. Measurements are based on 250µm wide strips of cortex spanning its full depth and include the total area of each strip, the area contained in each layer, the total number of neurons, astrocytes, and oligodendrocytes in each layer, and the size of each neuron. These measurements are adjusted for shrinkage effects, gliosis, and volume densities are calculated. This study will survey primary, association, and limitic cortices. Detailed information about the relative invariance of different cortical layers, different neuronal types, and gliosis is being collected. Data from the analysis of frontal lobe regions will be presented. (Supported by the Huntington’s Disease Society of America).

574.4

EVIDENCE FOR NEURONAL DEGENERATION IN THE SUBTHALAMIC NUCLEUS IN HUNTINGTON’S DISEASE. Karen M. Harrington, Robert L. Ferrante and Neil W. Kowall, Dept of Neuropathology and Experimental Neuropathology Laboratory, Massachusetts General Hospital, Boston MA 02114.

Recent anatomical, physiological, pharmacological and pathological evidence suggests that decreased excitation of the internal segment of the globus pallidus (GPi) by the subthalamic nucleus (STN) produces hyperkinetic movement disorders in man and primates. It has been further theorized that the hyperkinetic movement disorder in Huntington’s disease (HD) results from selective depletion of inhibitory enkephalinergic fibers in the external segment of the globus pallidus (GPe) which in turn leads to pathological disinhibition of inhibitory projections to the STN and thus the physiological equivalent of a STN lesion. Because we did not find evidence of disproportionate depletion of enkephalaminergic fibers in the STN in HD, we theorized that the basis for the hyperkinetic movement disorder may lie in the STN itself. We therefore examined the STN for evidence of neuronal degeneration in HD utilizing a polyclonal antibody against ubiquitin, a sensitive marker for neuronal injury and stress response. In a series of HD cases (n=9) of various grades the maximal number of ubiquitin positive cells in the STN was 147±33 per mm². In normal age-matched STN (n=5) the mean maximal density of ubiquitin positive cells was 57±29 per mm² (p<0.05). Our findings suggest that the STN is affected in HD. The basis for the hyperkinetic movement in HD may, therefore, lie in abnormalities of the STN itself rather than in the striatum or pallidum.
574.5 UBIQUITIN IMMUNOREACTIVITY IS INCREASED IN HUNTINGTON'S DISEASE CEREBRAL CORTEX. Abida I. Evans and Neil W. Korgenski. Neurology Service, Massachusetts General Hospital, Boston MA 02114.

Neurons respond to injury by increasing the synthesis of endogenous stress proteins such as ubiquitin which is conjugated to abnormal proteins destined for non-lysosomal proteolysis. In several neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis, neurons contain ubiquitin immunoreactive inclusions. In Huntington's disease (HD), specific subsets of neurons degenerate in the cerebral cortex and striatum without evidence of specific neuropathological changes produced by regional stress or injury in HD. In all HD cases, variable amounts of dot-like immunoreactivity were found in the superficial cortical layers and white matter identical to that seen in controls. In both low and high grade HD, scattered, diffusely ubiquitin positive neurons were found within the cortex and infracortical white matter. In one high grade HD case with severe cortical changes, ubiquitin immunoreactivity was especially prominent within diffusely stained neurons, intracytoplasmic inclusions and axonal spheroids. The presence of ubiquitin immunoreactivity within a subset of neurons in HD cortex suggests that abnormal proteins may accumulate during the degenerative process. The nature of these proteins and their contribution to neuronal degeneration is unknown but their identification may clarify the pathogenesis of HD.


Huntington's disease (HD) results in massive degeneration of neurons in the basal ganglia, but neuronal loss in HD cerebral cortex has not yet been clearly established. We studied cortical neurons in individual laminae of the dorsal frontal cortex in 5 HD (Vonsattel grade IV) and 5 age matched control monkeys. Significantly less neuronal loss was found in layer VI (HD 117±29 vs control 207±32, or greater than pc0.005), and layer V (pc0.05), and possibly layer III (13%, but no change in layer II and IV. Neurons in layers V and VI project to thalamus, claustrum, and other regions of cortical cortex, but not to the basal ganglia: thus their loss is unlikely to be the result of retrograde degeneration secondary to striatal pathology. We are currently performing autoradiography for glutamate receptor subtypes and immunocytochemistry for nitric oxide synthetase in order to explore possible neurochemical mechanisms underlying the neuronal loss.

574.7 EFFECTS OF CHRONIC STRIATAL LESIONS ON CORTICAL NEUROEPITHELIALS. S. Garside, I.C. Furtado and MF Mazurek. Department of Biomedical Sciences, McMaster University Medical Centre, Hamilton, Ontario, Canada.

We have previously reported widespread increases in CCK-LI as well as more localized changes in VIP-LI and NPY-LI in Huntington's disease (HD) postmortem cerebral cortex. It is not clear whether the results of abnormal feedback from the striatum or the result of independent cortical pathology. To investigate this issue we examined the effect of chronic striatal lesions on cortical peptide levels in the rat. Animals were given unilateral intrastriatal injections of QUIN (240nmol/saline or saline (1ul) and sacrificed by decapitation 9-12 months later. Two mm coronal sections incorporating the lesion were sliced from each brain and photographed. The striatum was removed from the posterior slice and photographed for neurochemistry. Strial SP-LI in QUIN animals was decreased by at least 50% as compared with controls. The lesion size as assessed with Blooapt II encompassed at least 60% of the striatum. CCK, SS, NPY and VIP levels were studied in 8 regions of cerebral cortex. No significant changes were found between QUIN and saline animals.

These results suggest that the widespread neuropeptide changes in HD cerebral cortex are independent of the striatal pathology seen in HD.

574.8 RELATIVE SURVIVAL OF STRIATAL PROJECTION NEURONS AND INTERNEURONS AFTER INTRASTRIATAL INJECTION OF QUINOLINIC ACID. K.D. Anderson and A. Fornage. Dept. of Anat. and Neurobiol. Univ. TN, Memphis. Huntington's disease (HD) is characterized by extensive loss of striatal projection neurons with no evident loss of somatostatin/neuropeptide Y (SS/NPY)-containing or cholinergic interneurons. Several studies have reported that intrastriatal injection of the NMDA receptor-specific excitotoxin quinolinic acid (QA) induces a pattern of neuronal death in rats that mimics HD, favoring the hypothesis that such excitotoxicity is involved in the neurodegenerative process in HD. Other investigators, however, have reported that striatal SS/NPY-containing neurons are vulnerable to QA. We conducted immunohistochemical studies in rats 2 months after injection of 50 nmol QA to determine the relative survival of 4 types of neurons as a function of distance from the injection site: 1) striatopallidal neurons labeled for enkephalin (ENK); 2) striatonigral neurons labeled retrogradely by fluorogold (FG); 3) SS/NPY-containing neurons; and 4) cholinergic neurons. By quantifying the number of cells in 0.2-mm wide concentric zones at increasing distances from the injection site, we were able to assess the relative vulnerability of each type to QA. Within the injection site, cholinergic neurons were normal in abundance, but ENK+, FG+, and SS/NPY+ neurons were sparse. Moving away from the injection site, the abundance of the latter three returned to normal in the sequence: ENK+, FG+, SS/NPY+. Thus, SS/NPY+ interneurons are more vulnerable to QA than are striatal projection neurons.

These results indicate that intrastriatal injection of QA does not induce a pattern of neuronal loss in rats that mimics HD, but the results do not necessarily refute the excitotoxin model of HD. NS-16360, NS-28721 (AR).

574.9 MULTINUCLEATED, TAU POSITIVE ASTROCYTOPHAGES IN PROGRESSIVE SUPRANUCLEAR (PSP) BRAIN TISSUE. T. Yamada, P. L. McGeer, W. G. McGee, Kinanen Lab., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

We reported earlier on Tau-2 positive oligodendroglial microtubular masses (OMMs)(Yamada and McGeer, Neurosci Letts 120:163, 1990) in some neurodegenerative diseases. We now report the occurrence of significant numbers of Tau-2 positive astrocytophages in PSP but not other neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. Tau-2 positive astrocytophages were seen in progressive supranuclear palsy (PSP), 17 Alzheimer disease (AD) and 13 Parkinson disease (PD) cases. Blocks of the midbrain were obtained from 9 Parkinson disease (PD), 4 progressive supranuclear parky (PSP), 17 Alzheimer disease (AD) and 13 age matched control cases. Blocks were sectioned at 15 μm and stained with cresyl violet. The number of pigmented cells per case was counted in every 25th section throughout the rostral-caudal length of the SNpc using a Quantimet 970 image analyzer (Cambridge Instruments). The mean number of neurons/section (Ntot al) was calculated for each case. The number of neurons/section was significantly lower in PD (mean = 89.2, sd = 84.4) and PSP (mean = 467, sd = 17.8) compared to AD (mean = 262.8, sd = 151.9) and controls (mean = 371.3, sd = 152.7) (ANOVA, F = 10.7). The mean number of neurons/section was also calculated from a subset of sections which contained the third cranial nerve (N3), where it exits from the mesencephalon. There were no significant differences between these two sets of sections. The number of neurons/section in the region of the third nerve provides a reliable estimate of the mean number of neurons/section throughout the SNpc. This method is reliable even when the degree of neurodegeneration is severe. The exit of cranial nerve III (oculomotor nerve) from the brainstem can be identified easily by observation of the vertical surface of the brainstem. This allows a readily identifiable, externally visible, anatomic landmark. (Supported by NIA 1P50AG05142 and the Veterans Administration).

Striatal-derived factors may influence outgrowth and survival of tyrosine hydroxylase-immunoreactive (TH-IR) neurons of the substantia nigra (SN) in vitro (Del Toso, et al J. Neuroscience, 1988). To examine whether striatal trophic factors may influence continued survival of dopaminergic SN neurons in the adult rat striatum in 250 µg/kg/day ibotenic acid (IBO) rats by stereotaxic unilateral injection of ibotenic acid, and after six weeks determined the number of TH-IR cells in the SN. TH-IR cells were counted bilaterally in lesion as well as in control animals.

The SN unilateral to the lesioned striatum had a consistent 10 to 15% decrease in the number of TH-IR cells when compared to the contralateral SN while intact animals showed a side to side difference of less than 4%. The number of TH-IR cells in the contralateral SN of lesioned animals was comparable to the unlesioned animals (16-18 x 10^3 cells per SN).

The results suggest that the absence of a trophic factor normally supplied by the striatum, but decreased in our lesioned striatum, is necessary for the continued survival or maintenance of TH expression in a population of dopamine cells in the adult SN. (Sponsored by the MRC of Canada)

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574.13 GENE MAPPING STUDIES IN FAMILIAL ALD. D.H.Donaldson, C. Huxham,* P.Sapp,* T.S.Barnes,* R.H.Brown, Jr, * and D. Patterson,* Dept. of Neurology, Univ. of Colorado Health Sciences Center, Denver, CO 80262; Eleanor Roosevelt Institute, Denver, CO 80206; Dept. of Neurology, Mass. General Hospital, Boston, MA 02118.

Multisite linkage analysis of 23 pedigrees with familial ALS suggests that a gene important in the disease might lie in the region Xq21-22.2 of human chromosome 21. Within this region lies the gene for superoxide dismutase, the trifunctional purine biosynthetic enzyme GPT, the interferon α receptor, and the interferon γ response element. We report the production of new probes for this region prepared from (CA) n-containing cosmids isolated from somatic cell hybrids that contain only this region of chromosome 21. We are constructing contig maps with these cosmids and YACs homologous to chromosome 21-specific DNA probes mapped to this region of 21, using somatic cell hybrids which allow dissection of this region of the chromosome into more than 10 parts.

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Immunoglobulin from ALS patients alters the electrophysiological parameters of rat skeletal muscle dihydropyridine-sensitive Ca++ channels (B. Srinivasan, et al J. Physiol., 1990). To determine whether Ca++ channels in ALS muscle are altered, subsarcolemmal membrane fractions enriched for transverse tubules were prepared from muscle biopsy specimens taken from ALS disease control, and normal control patients. These fractions were then assayed for the presence of dihydropyridine-sensitive voltage-dependent Ca++ channels (DHPRs) with 3H-PN-200/100. Results indicate an increased KD (1.17 ± 0.06 nM) in ALS muscle compared to normal and myopathic controls (high affinity KD ± S.D. = 0.17 ± 0.07 nM). In some ALS muscle specimens, there was also up to a 5-fold increase in the number of DHPR binding sites relative to controls, correlating crudely to the degree of denervation noted histologically. However, five non-ALS denervated muscle specimens (from one familial ALS, two spinal muscular atrophy, and two traumatically denervated control patients) yielded elevated Bmax values, but with KD values indistinguishable from those of ALS muscle. This difference in ALS muscle DHPR Kd is not solely explainable by denervation state, and may reflect changes attributable to immunological alterations of Ca++ channels in ALS.

Supported by the Muscular Dystrophy Association.

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Previously, we demonstrated that intraaxonal concentrations of K and Cl were increased significantly in tibial nerve of streptozocin-DB rats. We have now assessed the ability of GA treatment (mixed bovine brain) to modify DB-induced alterations of biochemical deficits caused by experimental diabetes (DB). Anesthesiology, Med. Sch., Stony Brook, NY 11794.

Ganglioside (GA) treatment corrects certain functional and biochemical deficits caused by experimental diabetes (DB). We have performed linkage analysis in 14 Filipino families with DB, a severe, X-linked neurological disorder of high penetrance which is characterized by adult-onset dystonia and parkinsonism. Previously, the XDP-Locus (DT3) has been assigned to Xq21 (Neurology 40:1438). Focusing on chromosomal region Xpll-Xq21, we studied 10 marker loci in DNA samples from a total of 94 family members, including 24 affected males. Highest LOD scores were 5.45, 4.52, 7.7, 4.4 for LocI DXS166, DXS159, DXS72, DXS367 at D=0.00 and 4.28 for PkRI at D=0.01. Analysis of linkage and linkage disequilibrium suggests that DT3 lies in proximity to DXS72 with DXS159 and DXS95 being flanking markers (Kupke et al in preparation). Presently, we are isolating from yeast artificial chromosomes highly polymorphic, (CA)n-based human DNA sequences close to DT3. Updated lod scores and data obtained with these probes will be presented.

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574.16 ACETYLC-L-CARNITINE COUNTERACTS EXPERIMENTALLY INDUCED DIABETIC NEUROPATHY. L. Pacifici*1, A. Beliucci*1, P. Piovesan*1, F. Maccari*1, A. Gori*1, A.M. Di Quìtto*1, M.T. Allegro*2, L. Ferrari*3, Sigma-Tau Institute for Research on Senescence, Pomezia, Rome, Italy; *Dept. Pharmacology, School of Medicine, Univ. of Milano, Italy; and *Sigma Tau Pharmaceuticals, Gallarate, MD 80000, USA.

Acetyl-L-Carnitine (ALCAR) exerts trophic effects on neurons. Here we report that similar effects were also observed in Sprague-Dawley rats, 3 months of age, in which a chemically-induced neuropathy was induced by a single injection of streptozocin 50 mg/kg i.v. or alloxan 100 mg/kg i.v. By means of a radioimmunoassay, an significant reduction (30-50%) of Met-enkephalin and substance P in the gut of diabetic rats was found. Such a reduction was successfully prevented by ALCAR administration (for 12 consecutive weeks) at the dose of 50 mg/kg/day i.p. or 300 mg/kg/day in drinking water. Treatment with ALCAR also fully prevented both the reduction on leukocytosis and reduced vascular conductance, as recorded on the sciatic nerve soleus muscle preparation, and the decrease (30%) in muscle contraction force. ALCAR also improved the motor performance on the Rotarod test. These data suggest for a potential therapeutic use of ALCAR in the treatment of diabetic peripheral neuropathy and further confirm ALCAR capability to ameliorate behavioral and morphological alterations of degenerative and age dependent disorders.

We have hypothesized that selected neurodegenerative diseases are due to altered DNA methylation in neurons. Fishman et al [Ann. Neurol. 1990]. Neurospecific gene expression in various species may be regulated in part by repetitive DNA elements known as ID (ident) sequences, which are transcribed by Pol III predominantly in brain. The prime ID element is BC-200. BC-200 is also expressed in cultured cells representing a variety of lineages. We have investigated the effect of agents that influence DNA methylation on BC-200 expression in four human cell types: two neural cell lines (H4, SH-SY5Y), and two non-neural cells (HeLa, primary skin fibroblasts). Cells were grown in medium containing 5-azacytidine (AZA, 3 μM), which inhibits CpG maintenance methylation (50 μM), a combination (50 μM) of the demethylating agent found in the neurotoxic Cyclic AMP (Gua) of Guam. Total RNA extracted by guanidinium-thiocyanate-phenol-chloroform was Northern-blotted with cDNA-probes labelled for BC-200, BC-185, and actin; the latter two RNA controls loading. Autoradiographs were quantitated by densitometry. BC-200 expression was detected in all four cell types, but was not apparently affected by treatment with AZA; this may reflect hypo-methylation of the element prior to AZA exposure. In contrast, MMAD decreased expression of BC-200 by 33-43% in neural cell lines (H4, SH-SY5Y), but not in non-neural cultures. Our data suggest that abnormal DNA methylation can alter BC-200 expression preferentially in neural tissue.

574.18 EXPRESSION OF WORLD WIDE MUTANT AND HUMAN PRION PROTEINS BY RECOMBINANT BACULOVIRUS INFECTED INSECT CELLS. D. Golgi*, R. Dhamija, R. P. Dooley, E. K. Fishman, J. Metuzals*, and K.P. Tewari. University of Texas Southwestern Medical Center, Dallas, TX 75395. Supported in part by the NINDS and the National Multiple Sclerosis Society.

A host protein encoded by the gene specifying the human scrapie amyloid precursor protein (Prion Protein, PrP; PrP gene) is linked with the development, and apparent attenuation, of the transmissible spongiform encephalopathies. Creutzfeldt-Jakob disease (CJD), Kuru, and Gerstmann-Sträussler-Scheinker disease (GSS). The extent and nature of point mutations in the human PrP gene is known to be associated with the occurrence of CJD and GSS. The wild type human PrP gene is expressed in human neural cells two days post-infection. PrP proteins were detected in the culture medium of infected cells indicating that the proteins were not secreted. The cell associated recombinant PrP proteins were detected in recombinant baculovirus infected insect cells two days post-infection. The recombinant baculovirus expression vector system provide an abundant source of prion proteins which can be used for comparative study and determination of the role of point mutations in the infectivity of prion proteins.

574.19 A CHICKEN PRION-LIKE PROTEIN UNDERGOES TWO POST-TRANSLATIONAL CLEAVAGES. D.A. Harris. Dept. of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, Missouri 63110.

We have previously identified a chicken prion-like protein (ch-PLP) as the major sequenceable protein in purified preparations of an acetylcholine receptor-inducing activity (D.A. Harris, et al., Neurosci. Abstr., 15:164, 1989). We have prepared stably transfected lines of mouse neuroblastoma (N2a) cells that express recombinant ch-PLP detectable by immunoblotting and immunoprecipitation. ch-PLP can be released from intact cells by incubation with bacterial phospholipase-C-specific inhibitor. c (PIPLC), suggesting that the protein is anchored to the cell surface by a glycosyl-phosphatidylinositol (GPI) linkage. Two forms of ch-PLP having molecular weights of 33-43kD and 10kD are also released into the extracellular medium in the absence of added enzyme. The 10kD fragment is derived from the N-terminals of the molecule since it reacts with an antibody raised against a synthetic peptide corresponding to pro-36 to pro-65 of the ch-PLP sequence. This fragment can be metabolically labeled with [3H]-proline but not with 3H-methionine suggesting that it is not extended beyond methionine. Ch-PLP is also detectable in a 100,000,000, membrane fraction of chicken brain, from which a 45kD form can be released by PIPLC. 33-43kD and 10kD forms are also detectable in a 100,000,000 supernatant fraction of brain. We propose that the 33-43kD ch-PLP molecule undergoes at least two posttranslational cleavages: one within or adjacent to the GPI anchor that releases the 33-43kD form, and a second near the middle of the protein that generates the 10kD fragment. The role of these cleavages in the deposition of amyloid in prion diseases is under investigation.

574.20 Ca2+-INDUCED SPONGIFORM FIBRILLATION IN SQUID GIANT NERVE FIBERS: A MODEL OF NEURODEGENERATIVE PROCESSES. H.M. Fishman, J. Metuzals* and K.P. Tewari. Dept. of the Texas Medical Branch, Galveston, "Univ of Ottawa, Ottawa, Canada.

Extensive vacuolation as observed by light and electron microscopy in stressed, isolated giant axons and surrounding Schwann cells of squid resembles spongiform changes seen in tissues of patients with neurodegenerative diseases (ND). Internal dialysis of unattracted axons with diacylpeptides containing Ca2+ (100 μM to 10 μM) induced vaculolation similar to that observed in stressed axon preparations with Ca2+ externally. These results suggest that neurodegeneration is mediated via a common mode of action involving intracellular Ca2+ regulatory processes.

Membranes involved in Ca2+ induced spongiform changes form calcium dependent glycocalyx with liposome containing membranes of vacuoles demonstrate regions of membrane damage, especially axolemma. Modified cytoskeletal filaments (10 to 20 nm diameter) are associated with these damaged regions. The large size of the squid giant nerve fibers and the relative ease with which morphological, biochemical and physiological methods are applied and integrated make giant axons of squid a unique preparation in which to study processes associated with degeneration.

Support: Office of Naval Research, Medical Research Council of Canada and American Health Assistance Foundation.

574.21 REDUCTION OF KAINATE-INDUCED SPECTRIN BREAKDOWN BY CALPAIN INHIBITORS. D. Evath, J. Estrada*, S.J. Mennerick, J. Dean, D.A. Harris. Dept. of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, Missouri 63110.

Excitotoxicity is associated with many neuropathologies, including ischemia, stroke, and epilepsy. The mechanism of excitotoxicity is thought to involve elevations of intracellular calcium, which in turn activates a potentially damaging enzyme cascade the activation of the calcium dependent protease calpain, which degrades intracellular proteins including the cytoskeletal component spectrin. Systemic administration of the excitotoxic glutamate analog kainate to rats produces an acute phase of convulsions and seizures followed by extensive cell death in the hippocampus and other brain regions. Cell death is dependent upon the induction of seizures, manifested externally by convulsions, in individual animals. The breakdown of spectrin is correlated well with the extent of cell death observed. Administration of Calpain Inhibitor [Ac-Leu-Leu-Nle-H], a potent inhibitor of calpain, reduced the breakdown of spectrin measured 4 days post-injection. The inhibition also reduced the extent of convulsions associated with kainic acid.

These data argue that Calpain Inhibitor 1 interferes with the cascade of events leading to cell death following excitotoxic injury. Calpain activation, which is reduced by the inhibitor, may be necessary for the infliction or maintenance of the cascade of events leading to cell death. Calpain inhibitors may thus represent a useful therapeutic approach to stroke and other forms of glutamate-related neurodegeneration.


The symptoms of multiple sclerosis (MS) can sometimes be relieved by a reduction in body temperature, by the administration of 4-AP, a potassium channel blocking agent. The improvement in symptoms has been attributed to a restoration of conduction to central axons in which conduction has been blocked by demyelination. To examine these possibilities in an in vitro mammalian system, demyelinating lesions were produced in the dorsal columns of fifteen adult rats by the intraspinal injection of chidium bromide under general anesthesia. Two chronically prepared animals were then examined at multiple time points, and the remaining animals were examined at autopsy. During the period of demyelination (2-6 weeks post-injection) a reduction of lesion temperature from 36°C to 32°C increased the number of axons conducting through the lesion by up to approximately 280%. The newly conducting axons had the long latency expected of demyelinated axons. However, in the same preparations the conduction of 4-AP, in a dose of 0.3-10 μM, had little or no effect on the number of conducting fibers.

In view of these findings, we suggest that conduction to blocked axons contributes to the improvement in symptoms observed with body cooling. It seems that the improvement caused by 4-AP may result primarily from other effects, possibly involving a reduction of central and neuromuscular synapses, thereby augmenting the action of pathways which are already functional. Supported in part by grants from the NINDS and the National Multiple Sclerosis Society.
**574.24**
EFFECT OF GM1 ON EXPERIMENTAL ENCEPHALOMYELITIS IN FEMALE LEWIS RATS
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Depart. of Anatomy & Cell Biology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107.
Experimental autoimmune encephalomyelitis (EAE) is a model for CNS associated autoimmune disease (e.g., multiple sclerosis). Fluctuations in clinical signs of EAE parallel changes in histamine in certain regions of the CNS. Administration of GM1 at low histamine levels or release from neurons or CNS-associated mast cells attenuate the course and severity of EAE. Since the ganglioside GM1 has been reported to inhibit histamine release from human and mouse mast cells, we investigated the effect of GM1 on the clinical course and severity of EAE. In all experiments adult female Lewis rats were inoculated to induce EAE and assessed for clinical symptoms beginning on day 7 post-inoculation. The effect of intraperitoneal injections of 50nmol/kg or equal volumes of saline from days 6 pi through day 10 pi on the clinical course of acute (experiment 1) and remission (experiment 2) EAE were investigated. Compared to Controls, only a slightly shorter duration of acute EAE was observed in GM1 treated rats and no effect was observed in the clinical course or severity of recurrent EAE. The last experiment assessed the effect of prolonged, chronic administration of GM1 or saline. Inoculations were made using an minipump on acute EAE. Pumps were implanted on day 6 pi and delivered an average daily dose of 30 mg/kg for 25 days (i.e., through day 31 pi). Again, no significant differences between experimental or control rats were observed for any of the clinical parameters assessed. We conclude that GM1 has no effect on the clinical course or severity of EAE in female Lewis rats.

Supported by: Fda S.P.A.

**SCHIZOPHRENIA**

**575.1**
DELAYED PHENCYclidine-INDUCED ALTERATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION IN RAT BRAIN.
X.M. Gao, O. Shirakawa, C.A. Tamminga,
University of Maryland at Baltimore, Baltimore, MD 21228.
Phencyclidine (PCP) is a psychotomimetic drug which produces psychotic symptoms in humans, closely resembling clinical manifestations in schizophrenia. PCP has been observed to produce delayed, reversible intracellular vacuolization after a single dose (Olney Science 244:1990). Because this action might be related to the long lasting psychotic manifestations of PCP, we studied the delayed regional metabolic alterations in rat brains following the administration of PCP, using glucose autoradiography. PCP (8.6 mg/kg) was administered 3,6, and 12 hours prior to the 14C-glucose experiment. At 6 hours after intraperitoneal administration, we observed PCP on thin light microscopic sections in the posterior cingulate cortex, hippocampus, and subiculum. The 14C-glucose uptake was decreased selectively in parietal limbic regions with vacuolizations (e.g., posterior cingulate, retrosplenial cortex, amygdala, ventral tegmental area) and, in addition, in auditory regions (p=0.001) and in visual cortex (p=0.03). However, no metabolic changes remained either in the neocortex or the extrapyramidal areas, and vacuolizations were not present. Glucose metabolism was decreased in the lateral habenula, an action opposed to that produced by antipsychotic drugs. The overall distribution of altered glucose utilization observed in this study, is distinct from the acute actions of PCP (Tamminga et al. Synapse 1:1987) in its restricted limbic effects. This may be a more precise animal model for psychosis because of the extended time course of the psychotomimetic action of PCP. Moreover, we have previously observed an altered distribution of regional metabolism in schizophrenia precisely in limbic areas, similar to the effects of delayed PCP.

**575.2**
CEREBRAL METABOLIC ACTIVITY AND ABNORMAL EYE MOVEMENTS IN SCHIZOPHRENIC PATIENTS.
Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228.
Schizophrenic patients have abnormal smooth pursuit and saccadic eye movements, and it is likely that these deficits are associated with abnormalities in suprana-brain stem oculomotor areas. We hypothesize that these abnormal eye movements are associated with abnormal metabolism in the frontal eye fields (FEFs) and/or supplementary motor area (SMA).

To test this hypothesis, we examined regional cerebral metabolic rates of glucose utilization (rCMRglu) with PET/ FDG in these areas and correlated them with measures of eye movements in schizophrenic patients.

- **Enzyme Measurements**: With the PET images, regions of interest for the FEFs and SMA were defined by stereotactic atlas coordinates reported by Fox et al (1 Neuropsychopharmacol. 5:348-358, 1991). Twelve of the 13 subjects had an MRI scan of the brain which was used to determine the PET slices of interest. Eye movements were tested with infrared oculography, and tasks included smooth pursuit and an antisaccade paradigm. The measure of the latter is distractibility, which is defined as the percentage of trials in which the patient makes a reflexive saccade inappropriately toward the target. The results show that saccadic eye movement distractibility correlates with decreased metabolism in the frontal eye fields (<0.58, p=0.05) and supplementary motor area (<0.64, p=0.02). Distractibility did not correlate significantly with metabolism in non-oculomotor areas. Other oculomotor measures did not correlate significantly with metabolic values. These preliminary results indicate that increased saccadic eye movement distractibility in schizophrenic patients results from a failure of FEFs and SMA to inhibit lower centers of oculomotor control.

**575.3**
HIPPOCAMPAL EFFERENTS TO THE NUCLEUS ACCUMBENS MODULATE SENSORIMOTOR GATING OF STARTLE IN THE RAT.
S.B. Caine, M.A. Geyer and H.R. Swerdlow*.
Departments of Psychiatry and Neuroscience, UCSD School of Medicine, La Jolla, CA 92037-0801.
"Prepulse inhibition" (PPI) occurs when the startle response to a startling stimulus is reduced by the prior presentation of a weak lead stimulus. PPI is an operational correlate of PCP. Moreover, we have previously observed an altered distribution of FEFs and SMA to inhibit lower centers of oculomotor control.

- **PET/ FDG Imaging**: In EAE, there is an autoimmune reaction against gangliosides which destroys the myelin sheath. This results in a inflammatory process, edema, and neurolysis. However, the exact mechanism of how this process occurs is not well understood. However, in vitro autoradiography has been used to determine BBB compromise. U-74006F treatment significantly reduced the incidence of clinical symptoms from 81% in the untreated animals to 44 and 56% with 3 and 10 mg/kg, respectively.

- **MRI Imaging**: In EAE, there is an autoimmune reaction against gangliosides which destroys the myelin sheath. This results in a inflammatory process, edema, and neurolysis. However, in vitro autoradiography has been used to determine BBB compromise. U-74006F treatment significantly reduced the incidence of clinical symptoms from 81% in the untreated animals to 44 and 56% with 3 and 10 mg/kg, respectively.

**575.4**
CHRONIC HALOPERIDOL AND CLOzapine TREATMENT AT THE CENTRAL AMYGDALA NUCLEUS.
The dopaminergic cell bodies of the ventral tegmental area (A) project to the central nucleus of the amygdala. The metabolic activity of the dopaminergic system in this area is not affected by amphetamine treatment. In vitro autoradiographic recording from the amygdala slice was used to assess the effects of chronic neuroleptic treatment. At 25 weeks of 1 mg/kg/day s.c. haloperidol, all rats displayed vacuous chewing movements, catalepsy and stereotypes. After 6 weeks chronic treatment with clozapine at 25 mg/kg/day i.p. all rats displayed passage and apathy. Haloperidol and clozapine did not produce significant changes from control in passive membrane properties: depolarization block was not induced. Haloperidol produced a heightened response to superfusion with muscarine and quisqualate. Clozapine dramatically altered the central nucleus response to dopamine (1 μM) superfusion; producing membrane depolarization, an increase in spontaneous firing, a blockade of the t-AHP and a broadening of the action potential. These data suggest that clozapine's antipsychotic efficacy may be mediated by a modulation of dopaminergic transmission at the amygdala level and that haloperidol's antipsychotic efficacy may be mediated by a modulation in excitatory transmission. (Supported by Scottish Rite Schizophrenia Research Program)
ALPH A2 ADRENERGIC BLOCKADE WITH YOHIMBINE DISRUPTS following variables: age, gender (all male), race and parental socioeconomic transmission in impaired sensory gating, the presynaptic alpha, adrenergic antagonist, yohimbine, was administered. Several doses were injected. To determine the dose response to the first click and an elevation of the response to the second. To determine if dopaminergic blockade could effect this alteration in gating, yohimbine was co-administered with yohimbine. Preliminary data indicate, that rather than ameliorating the effects of yohimbine, D1 blockade actually exacerbates the gating deficit. (Supported by P50 MH44212-02)


The relationships of cognitive functions and putative measures of cerebral atrophy were studied in schizophrenics and normal males 50 years of age or younger. Visual ratings of cerebral spinal fluid (CSF) surrounding Frontal, Parietal/Occipital, and Temporal lobes were made by two raters blind to diagnosis on coronal images. Volumes of ventricles were obtained using semi-automated tracings (see Flaum et al., this conference). Neuropsychological tests were administered to obtain summary scores for IQ and other cognitive domains. Schizophrenics (N=54) met DSM-III-R criteria and had no concurrent medical illness or history of substance abuse. Controls (N=33) were recruited and were comparable in age, race, socioeconomic status. All subjects were scanned on a 1.5 Tesla GE Sigma MR scanner, with 5 mm cranial slices and a 2.5mm gap. All tracings were performed by a single rater, blind to diagnosis, using a semi-automated method. Intrarater reliability was consistently high, and inter-rater reliability was acceptable (kappa >0.70) for all structures except for the amygdala and thalamus. Subarachnoid CSF volume was estimated based on subjective ratings using a 0-5 scale.

Results: Using two tailed t-tests, schizophrenics had a significantly larger cranial volume, and left cerebral ventricle, and left thalamic volume than controls. Ratings of subarachnoid CSF were higher in the schizophrenic sample. Third ventricle volume was significantly larger in the schizophrenic group. All of these differences remained significant (p <0.05) after cranial volume and age were covaried. There were no significant differences in the volumes of the following structures: lateral ventricles, caudate, putamen, globus pallidus, hippocampus, amygdala or cerebellum. Conclusions: The finding of smaller cranial and cerebral volume in schizophrenia is replicated, even after carefully controlling for socioeconomic factors, suggesting an early developmental defect. The findings of smaller cerebral volumes and increased subarachnoid CSF, even after cranial volume is covaried, suggest that an atrophic process may also be considered.

ALPHA, ADRENERGIC BLOCKADE WITH YOHIMBINE DISRUPTS AUDITORY SENSORY GATING IN RATS. K.E. Stevens, J. Melzer, S.L. Stryker and G.M. Rose. Department of Pharmacology, UCHSC and Medical Research, VAMC, Denver, CO 80262.

In medicated rats and normal humans, midauditory transmission evoked potentials recorded in response to the second of a closely spaced pair of clicks were reduced compared to the first. Amphetamine and rats of rats or humans was similar to that seen in schizophrenics. Animals studies using selective antagonists have implicated alterations in both noradrenergic and dopaminergic neurotransmission in this phenomenon. To more directly assess the role of noradrenergic transmission in impaired sensory gating, the presynaptic alpha, adrenergic antagonist, yohimbine, was administered. Several doses were injected. To determine the dose response to the first click and an elevation of the response to the second. To determine if dopaminergic blockade could effect this alteration in gating, yohimbine was co-administered with yohimbine. Preliminary data indicate, that rather than ameliorating the effects of yohimbine, D1 blockade actually exacerbates the gating deficit. (Supported by P50 MH44212-02)


In a preliminary study by our group (see O'Leary et al., this conference), 54 male patients with schizophrenia were found to have significantly more cortical CSF than 33 male controls in frontal and temporal regions. A visual rating method was used to rank MRI scans by the amount of cortical CSF seen in the subarachnoid space.

To validate the visual rating method and to begin to understand the etiology of increased cortical CSF, two computerized methods were used on coronal MRI slices (forward of the anterior horns of the lateral ventricles). Using imaging analysis software developed in our laboratory, inner cranial and cerebral surfaces were semi-automatically traced, and the surfaces were used to define three-dimensional volumes. Ratios of peripheral cortical, ventricular and cranial volume were calculated and were found to be significantly correlated with the visual ratings for the same subjects (r=0.29, p<0.01). Additional tracing data suggest that the increased cortical CSF results, in part, from sulcal widening. A segmentation algorithm was used to classify MRI voxel intensities as gray matter, white matter, or CSF for each subject's series of frontal lobe slices. Ratios of the number of voxels classified as CSF to the total number of voxels classified were calculated. These ratios were found to be significantly correlated with the visual ratings (r=0.15, r=0.57, p<0.05) and with the tracing ratios (r=0.16, r=0.67, p<0.01). Gray matter and white matter volume changes which correlate with increased cortical CSF are being explored. The comparison of three different methodologies used to evaluate MRI scans serves to validate each, and each may prove useful in generating hypotheses concerning brain changes in schizophrenia and schizophrenic subtypes.


Shizophrenics had significantly higher ratings of CSF surrounding the Frontal and Temporal lobes and had significantly larger third Ventricle volumes than normals. These findings remained when age was covaried. IQ showed no relationship to any CSF measure. In both normals and patients, significant correlations were found between CSF measures and long- and short-term verbal memory, visual memory, and attentional measures. The effects of age differed in schizophrenics and normals but significant correlations remained in both groups when age effects were controlled. Future studies will investigate whether increased CSF reflects changes in gray or white matter using semiautomated segmentation techniques (see Keeler et al. this conference for discussion of computer methods of MRI analysis).

The growth of axons and formation of synaptic connections are associated with the expression of the neuronal membrane phosphoprotein, GAP-43. To examine whether GAP-43 levels were altered in conditions of abnormal brain development such as in schizophrenia, we compared the levels of GAP-43 and other synaptic proteins in postmortem brain tissues derived from control and schizophrenic subjects. Following in vitro phosphorylation reactions in the presence of either Ca2+ or CaM, synaptic protein phosphorylation was detected from primary visual (A17), and visual association (A20) areas were analyzed by two-dimensional gel electrophoresis and fluorography. Quantitative analysis revealed that levels of phosphorylated GAP-43 were increased in all regions examined, whereas the levels of phosphorylated tauulin and another major synaptic protein showed no change. Preliminary analysis of the frontal cortex (A10/11) in schizophrenia brains also showed increased levels of GAP-43 phosphorylation, suggesting that the anomaly observed in the previous studies is not limited to visual cortical areas. Finally, the fact that halldol-treated rats did not show any significant increase in GAP-43 phosphorylation suggests that our findings in schizophrenic brains are not the result of neuroleptic treatment and that an altered expression of GAP-43 and other synaptic proteins may correlate with some aspect of the disease. Supported by the Scottish Rite Schizophrenia Research Program.

575.12 ABNORMALITIES IN NAAG AND NAALADase LEVELS IN SCHIZOPHRENIC BRAINS. H. Shibuya, M. Morii* and M. Toru*. Dept. of Neuropsychiatry, Dept. of Pharmacology, and Dental Univ., Tokyo 113 JAPAN.

The pharmacological profile of sigma receptor in mizar rat and human brain was studied using [3H]-, (2-0-tolyl) guanidine([3H]-DTG). Furthermore, sigma receptor binding in the postmortem brains of 10 controls and 12 schizophrenics were assayed. None specific binding was determined in the presence of 10-5 M haloperidol. 100nM [3H]-DTG significantly decreased [3H]-DTG binding in rat brain homogenate but not in human brain tissue. Kt, Km*, Ks* made no obvious difference in the binding between two species. Liv significantly increased [3H]-DTG binding in the human brain cortex but not in the rat brain.

In schizophrenics, sigma receptors in the frontal cortices, the parietal cortices and the occipital cortices showed no obvious difference compared to those in controls. There was no significant difference in sigma receptor binding between drug groups and off-drug groups in schizophrenics. These findings suggest that sigma receptor might not play important roles in the pathogenesis of schizophrenia.


In order to consider whether behavioral differences in monozygotic twins discordant for schizophrenia (MZDS) are associated with neuroanatomical differences, we measured the surface area of 27 cortical regions using a two-dimensional computer-generated reconstructions of magnetic resonance (MR) images. Four brains from each MZDS group were studied using DSM-III-R criteria for the diagnosis of schizophrenia. Monzygosity was determined by blood red cell marker assays and a standardized questionnaire. Contiguous non-skipped 1.5T-weighted T2 images were obtained in the axial plane. For each of the 27 regions of interest (ROIs), differences in surface area (SA) within twins pairs and differences in SA between unrelated twin pairs were compared using a repeated measure ANOVA and Bartlett's test to compare variances. In a twin study (Gazanzu et al. 1991), 15 of 27 ROI SAIs in the left hemisphere of five normal female MZ twin pairs showed significantly less variance. In the MZDS group, only one ROI showed significantly less variance in the left hemisphere.

In a twin study of normal MZ twin pairs showed only four areas of significantly less variance. In the MZDS group significantly less variance was seen in three of these same regions. These preliminary results are consistent with the view that 1) schizophrenia reflects disease of the left hemisphere; and 2) the pathogenesis of the disease goes beyond abnormalities of neurotransmitter function and involves disorganization of cortical structures. (Supported by NARSAD, and ONR N00014-89-J-3035.)

575.14 SENSORMOTOR GATING OF THE STARTLE REFLEX (SR) IS MEDITATED BY VENTRAL PALLIDAL EFFERENTS TO THE PEDUNCULOPONTINE NUCLEUS. N.R. Swerdlow*, S.B. Caine and M.A. Geyer, Departments of Psychiatry and Neurosciences, UCSD School of Medicine, La Jolla, CA 92039

The SR is inhibited or "gated" when a startling stimulus is preceded 30-500 msec earlier by a weak click or "prepulse." This "prepulse inhibition" (PPI) provides an operational measure of sensorimotor gating. PPI is impaired in schizophrenic patients, and this loss of startle gating may be a psychophysiological correlate of deficient inhibitory processes that cause sensorial and cognitive "flooding" in schizophrenia.

Our previous findings indicate that PPI is modulated in rats by neural circuitry connecting the hippocampus, nucleus accumbens and ventral pallidum (VP). Davis (J. Neurosci. 1982) reported that the "primary" control of the SR occurs in a 5-neuron circuit that links the sensory receptor through the pontine reticular formation to the motor effector. We examined how limbic/motor circuitry that modulates PPI might influence activity in this "primary" startle circuit.

In a replication of our published work, infusion of picrotoxin into the VP disrupted PPI of acoustic startle. PPI, but not startle itself, was profoundly impaired in rats after electrolytic lesions of VP efferent to the pedunculopontine nucleus (PPN). PPI was not significantly impaired by electrolytic lesions of the dorsomedial thalamus. Limbic/motor information that modulates PPI appears to access the "primary startle circuit" via ventral pallidal efferents to the PPN. By linking forebrain and reticular formation substrates that modulate the SR, we have identified circuitry that controls a specific quantifiable behavioral abnormality in schizophrenia.


Recent studies suggest that disturbed glutamatergic neurotransmission may play a role in schizophrenia. An important contribution to this hypothesis is the observation that phencyclidine (PCP), a potent psychotomimetic drug, acts at the NMDA-type glutamate receptor and at the N-Methyl-D-Aspartate (NMDA)-Aspartyl-Glutamate (NAAG), has been proposed to act as a mixed agonist-antagonist at the NMDA receptor. To determine whether NAAG is involved in the neurochemical alterations of schizophrenia, NAAG and its catalytic enzyme, N-Acetylated Alpha-Linked Acidic Dipeptidase (NAALADase), were measured in eight different regions of freshly frozen schizophrenic and control brains. Preliminary results indicate abnormalities in NAAG levels and NAALADase activity in schizophrenics compared to normal controls. NAAG levels were increased in frontal and parietal cortices, but decreased in putamen. NAALADase activity was decreased in temporal and frontal cortices, hippocampus and amygdala. The alterations of NAAG in GAP-43 in both regions 17 and 20 of schizophrenic brains were about 3-fold higher than in control brains. A similar increase was observed for other Ca2+-dependent and CaM-dependent synaptic phosphoproteins, whereas the levels of phosphorylation for tubulin and another major synaptic protein showed no change. Preliminary analysis of the frontal cortex (A10/11) in schizophrenia brains also showed increased levels of GAP-43 phosphorylation, suggesting that the anomaly observed in the previous studies is not limited to visual cortical areas. Finally, the fact that halldol-treated rats did not show any significant increase in GAP-43 phosphorylation suggests that our findings in schizophrenic brains are not the result of neuroleptic treatment and that an altered expression of GAP-43 and other synaptic proteins may correlate with some aspect of the disease. Supported by the Scottish Rite Schizophrenia Research Program.

575.16 IN VIVO 1H MAGNETIC RESONANCE SPECTROSCOPY (MRS) OF THE HIPPOCAMPUS/AMYGDALA COMPLEX IN SCHIZOPHRENIA. N.A. Nasrallah, T.S. Wyer, J.T. Fisch, A. Krampe, T. S. Schmeltroock*, A. O'Brien* and P.M.,. Robitaille*, Departments of Psychiatry and Radiology, Ohio State University, Columbus, Ohio 43210

Several postmortem and brain imaging studies provide evidence for pathology of the medial (limbic) temporal lobe in schizophrenia. This includes hypoplasia and histopathological abnormalities in the hippocampus of schizophrenic patients, suggestive of neurodevelopmental disruption of neuronal migration. We conducted an in vivo MRS study of the medial temporal lobe in schizophrenics and controls to generate hypothesis about the pathological abnormalities in normal neurochemical functions of this region in schizophrenics.

Eleven schizophrenic patients (by DSMIII-R) and six healthy volunteers were included in the study. In 18 MRS was conducted using a GE Sigma (1.5T).

Proton spectra were acquired from a 2 X 2 X 3 cm3 voxel localized in the right and left hippocampus and amygdala using a modified SE sequence with a TE of 1500ms. Signal intensities of N-acetyl aspartate (NAA), creatine, and choline relative to a standard compound representing the normalized intensity in the hippocampus and amygdala were also observed in the neuroleptic control group, suggesting that these changes may be the effects of drug treatment. These results support the hypothesis that glutamate may be involved in schizophrenia and underscore the importance of studying the metabolism of NAAG, a potential NMDA ligand, in the pathophysiology of schizophrenia.

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ERPs were recorded from 19 normal and 18 schizophrenic adult subjects on a visual discrimination task, the Span of Apprehension (Span) which may detect vulnerability factors in schizophrenia. An RT condition preceded the span (identical stimuli, letter arrays) was presented in both conditions. Subjects responded to the visual arrays in the RT condition and discriminated between the presence of either a T or F in the Span condition. EEG was recorded from 17 scalp sites and ERPs spanned a 2 second epoch. Difference potentials (Span-RT) were computed to isolate slope differences in the 2 conditions. topographic fields were computed at the peak of SEN.

Schizophrenics produced significantly less SEN than normals. SEN differed in topography and time course from the exogenous components (N1, P2) it overlaps. SEN is similar to NA (Ketter et al 1983). which is associated with attentional effort during pattern recognition. Results suggest that schizophrenia is impaired in allocation of adequate attentional resources for span processing. This deficit is manifest early (220 msec) in discriminative processing. SEN results are similar to those obtained from normal and schizophrenic children.

AFFECTIVE ILLNESS AND RELATED DISORDERS

ANXIETY RESPONSES TO IV MCPP IN HEALTHY SUBJECTS AND PATIENTS WITH PANIC DISORDER. A.W. Goddard, D.S. Charney, M. Germain, G.R. Heninger, and S.W. Woods; Yale Univ. Dept. of Psychiatry, New Haven, CT 06519.

Patients with panic disorder (PD) have been reported to have an increased sensitivity to oral doses of the 5-HT agonist, m-chlorophenylpiperazine (MCPP). However a previous study by our group failed to find patient vs healthy subject behavioral differences following administration of higher doses of intravenous (IV) MCPP. The current study used a lower dose of IV MCPP, 0.05mg/kg, to determine whether the different findings were due to a dose effect. METHOD: Twenty-nine PD patients and 22 healthy subjects participated in two study days, separated by at least one week. They received either a bolus injection of normal saline or a solution of 0.05mg/kg MCPP, according to random assignment. Within challenge measurements included visual analog scale (VAS) anxiety, DSM III-R panic symptomatics, and blood pressure and heart rate (HR). Baseline VAS anxiety on the active challenge day was 41±28 mm for the PD group and 41±11 mm for the healthy subject group. These baseline values were virtually the same for the placebo study day. Inspection of the data revealed that there was no effect at 30 min, post infusion. The MCPP minus placebo change from baseline anxiety at 30 min was 26±30 mm for the PD group and 17±27 mm for the healthy group. This difference was not significant on patient vs healthy subject comparison. While 5-HT may play an important role in mediating human anxiety responses in general, it is unlikely that anxiety associated with PD specifically is the result of 5-HT receptor hypersensitivity. The effect of different routes of MCPP administration remain to be investigated.

INCREASED SENSITIVITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS TO GLUCOCORTICOID FEEDBACK INHIBITION IN A TRANSGENIC MOUSE MODEL OF DEPRESSION TREATED WITH ANTIDEPRESSANTS. N. Barden, M-C. Pepin and F. Pouliot; Molecular Psychogenetics, CHUL Research Centre and Laval University, Ste-Foy, Québec, Canada G1V 4G2

We have created transgenic mice which bear a glucocorticoid receptor mRNA antisense transgene as an animal model for depression. These animals have decreased glucocorticoid binding and corticosterone expression, we have treated transgenic mice with desipramine (20mg/kgBW) and observed the effects on GR mRNA, glucocorticoid binding and corticosterone. When we restored the sensitivity of the HPA axis to glucocorticoid feedback inhibition by treating with CHCl, heat (56°C, 60°C or 90°C), and protease (proteinase K or trypsin), but not destroyed by 4°C incubation at 4°C and RNase. Growth of the cells for 24 hr in serum-free medium increased the biological activity approx. 100-fold compared to regular 10% FCS containing media. On a Percoll density gradient of culture media, the activity migrated to 1.081 approx. 100-fold compared to regular 10% FCS free medium increased the biological activity.

EFFECTS OF SODIUM LACTATE INFUSIONS ON CISTERNAL LACTATE AND CARBON DIOXIDE MEASURES IN NONHUMAN PRIMATES. J.D. Coglan, L. Leonard, M. Anderson, M. Blanken, A. Zea, S. Friedman Phillip, T. Bassoff, M.A. Magari, M.D. from: New York State Psychiatric Institute, Biological Studies Unit, Columbia University and Primate Behavioral Facility, SUNY Upstate.

To further understand lactate induced panic in patients with panic disorder, we evaluated the central effects of sodium lactate infusions as measured by cisternal lactate and gas measurements in a sample of nonventilated nonhuman primates that were under ketamine anesthesia. We hypothesized that development of the characteristic peripheral biochemical effects of infused sodium lactate, no increases of central lactate or carbon dioxide was observed. The study therefore supports previous findings of non permeability of the blood brain barrier to lactate but conflict with other nonhuman primate studies suggesting a central increase in lactate during infusions. These discrepant data may be accounted for by type of anesthetic agent used, ventilation effects and concentration of lactate infused. Available studies, however, are consistent regarding the absence of central hyperventilation during lactate infusions, a factor hypothesized to be an important trigger of an abnormally sensitive "suffocation alarm mechanism" in panic disorder.

Characterization of a growth promoting agent for human neuroblastoma cells derived from schizophreinic CSF. S. Shihabuddin, J.P. Schwartz, and J.B. Stevens; 1) NIMH, St. Elizabeths Hosp., Washington DC 20032, & 2) CNB, NINDS, NIH, Bethesda MD 20892.

We have identified a factor in schizophrenic (Sch) but not control CSF which promotes the growth of the human neuroblastoma cell line SH-EP. Once transformed, the cells themselves produce the factor. Further characterization has shown that the biological activity is destroyed by treating with CHCl, heat (56°C, 60°C or 90°C), and protease (proteinase K or trypsin), but not destroyed by 4°C incubation at 4°C and RNase. Growth of the cells for 24 hr in serum-free medium increased the biological activity approx. 100-fold compared to regular 10% FCS containing media. On a Percoll density gradient of culture media, the activity migrated to 1.081 approx. 100-fold compared to regular 10% FCS free medium increased the biological activity.

Specific high-affinity binding sites for [H]-imipramine (IMI), that label the serotonin (5-HT) reuptake site of monoamine neurotransmitters in brain and platelets, have been reported. In studies reported by our group and others, drug-free depressed patients have exhibited a reduced number (Bmax) of platelet IMI binding sites when compared to normal controls. Other groups have reported a seasonal variation in platelet IMI binding kinetics that masked any group differences in IMI Bmax.

With our large database (104 controls, 178 depressed patients, 88 non-depressed psychiatric patients), we examined the possibility of monthly variations in platelet IMI binding site density or affinity using one-way ANOVA. We observed no significant monthly variation in platelet IMI binding Bmax or Kd in controls or patients with major depression. There were small statistically insignificant peaks of IMI binding in February and August with the lowest binding found in July. Bmax values for depressed patients were significantly below the monthly mean of normal controls Bmax values at every time point examined. There were no differences between these populations. Cortisol levels were also assayed as a nonspecific endocrine measure of stress. Clonidine induced a six-fold increase in plasma growth hormone levels in both patients and controls. There was no difference in the amount of stimulation between these populations. Cortisol levels dropped by 15% during the period of clonidine-induced growth hormone stimulation and there was no significant difference between patients and controls.

576.7 EFFECTS OF LITHIUM ON TYROSINE HYDROXYLASE GENE EXPRESSION IN PC12 CELLS. G. Allen and N.C. Banoop. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.

Lithium is an effective drug for the treatment of some manic disorders, but its precise mechanism of action has not yet been determined. It has been shown to affect the insulin-like gene via inhibition of insulin-like growth factors and to affect the coupling of G-proteins. Recent evidence showing that lithium augment c-fos expression in PC12 cells has led to the suggestion that lithium might also act at the level of gene expression. The control enzyme of catecholamine biosynthesis, tyrosine hydroxilase (TH), has been shown to be a target of the AP-1 transcriptional activator complex. PC12 cells were treated with nerve growth factor (NGF) and/or lithium and TH mRNA levels were determined by Northern analysis. Lithium (0.1 mM and 1 mM) elevated TH message levels in NGF-treated cells after 5 and 6 days, but not after 8 days. TH mRNA levels in undifferentiated cells were not affected. Ten millimolar lithium decreases TH mRNA levels with respect to controls in both differentiated and undifferentiated cells after 5 and 8 days of exposure. These data suggest that chronic lithium treatment can affect TH gene expression in this cell line, but at the highest concentration, might have nonspecific effects. We are currently studying this system in more detail, since it provides a model to study its action in neural cells. (Supported by NINDS NS23062)

576.8 AN HYPOTHESIS OF THE NEUROBIOLOGY OF THE ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD) SYNDROME. W.A. Weinberg and R.A. Brumback. Dallas Children's Hospital, Dallas, TX and University of Oklahoma, Oklahoma City, OK.

Children and adolescents not doing well in school and/or home are often labeled as suffering from ADHD. However, ADHD is a symptom complex resulting from multiple specific etiologies. We have developed an hypothesis in which ADHD symptoms are explainable as genetically-based dysfunction of specific brain areas. Depression relates to loss of right parietal-temporal cortical control of locus ceruleus and median raphe nuclei with resultant reduced bioamine neurotransmission to cerebral cortex bilaterally. Disturbed control of hypothalamic circadian functions results in normal sleep. Primary disorder of vigilance relates to loss of controlling modulation by inferior parietal lobe on reticular activating system resulting in sleepiness and narcolepsy, inferior parietal lobe control is intact, but genetically-abnormal reticular activating system aberrantly influences both cerebral hemispheres with resultant clinical symptoms of affective illness. Disorder of right hemisphere not only generates depression, but its influence on left hemisphere can result in mixed symptoms (depression and mania) or (less commonly) pure mania. Occasionally, primary dysfunction of left hemisphere (limbic) cortex can induce right cerebral hemisphere dysfunction preventing clinically as mixed symptoms or as pure major depression. Individual variation in higher cortical cognitive functions may be apparent as learning disabilities. In right parietal cortex learning disability syndrome, stressing dysfunctions cortical areas in an unfavorable learning environment results in inattention, secondary hypovigilance, and promotion of affective illness. Although most symptoms of conduct disorder are secondary to underlying affective illness, dysfunction of anterior/interior right frontal cortex results in primary conduct disorder (sociopathy). Comorbidity and concurrent diagnoses are explainable as a consequence of interactions between affected brain centers.
Rats forced to swim with no possibility of escape initially swim vigorously but soon show considerable immobility. In contrast, animals tested in soiled water exhibited almost no immobility. We hypothesized that behavior in soiled water resembles a panic-like response, which will respond to treatment with antipanic effects. The present results supported this hypothesis by showing that buspirone, diazepam, and imipramine had no significant effects on immobility when rats were tested in fresh water. In soiled water, diazepam was still ineffective; imipramine increased immobility slightly; and buspirone increased immobility to the level of animals tested in fresh water. Since the selective serotonin agonist buspirone has relatively greater antipanic effects clinically than diazepam or imipramine, we concluded that our variation of the forced swim test may be a useful animal model for studying mechanisms of panic and treatment of panic disorder. Supported by P01 AA07670 and R01 AA06721.

CALCITONIN GENE-RELATED PEPTIDE (CGRP) RELEASE IN RESPONSE TO ETHANOL STRESS: IN VIVO AND IN VITRO STUDIES. D.A. Hinks, T. Callaghan, and D.H. Wilkie. Dept. of Pharmacology, University of Melbourne, Parkville, Vic. 3052.

Rats were tested continuously in fresh water or subchronically in ethanol-stressed (1 g/100 g bw) water for 2 weeks; VMH and PVN were freeze-clamped after 1, 3, 5 or 7 days. In addition, some rats were tested for 5, 6 or 7 days with an ethanol withdrawal period of 2 days prior to testing. CGRP release increased for VMH and PVN at 1 day and was increased for VMH by 3 days. CGRP release was comparable for VMH and PVN at 3, 5 and 7 days, with VMH having the highest levels. CGRP release was decreased at 7 days for VMH following withdrawal. These results suggest that CGRP is involved in the adaptive response to ethanol stress, possibly through the hypothalamus.

Selective serotonin receptor agonists (5-HT1A) may counteract the effect of ethanol withdrawal. The present study was to examine the effects of chronic ethanol on the release of CGRP in the VMH and PVN of rats. Rats were assigned to 7 groups: control (C), and groups receiving chronic ethanol (E: 0.5 g/kg in drinking water during 3 days), and chronic ethanol withdrawal (E: withdrawal 3 days). CGRP release was measured in vivo and in vitro. In vivo, CGRP release was increased in the VMH and PVN of rats following withdrawal (E: withdrawal 3 days), but not in VMH of rats following chronic ethanol (E: 0.5 g/kg in drinking water during 3 days). In vitro, CGRP release was decreased in the VMH and PVN of rats following withdrawal (E: withdrawal 3 days), but not in VMH of rats following chronic ethanol (E: 0.5 g/kg in drinking water during 3 days). These results suggest that CGRP may be involved in the adaptive response to ethanol withdrawal.

Selective serotonin receptor agonists (5-HT1A) may counteract the effect of ethanol withdrawal. The present study was to examine the effects of chronic ethanol on the release of CGRP in the VMH and PVN of rats. Rats were assigned to 7 groups: control (C), and groups receiving chronic ethanol (E: 0.5 g/kg in drinking water during 3 days), and chronic ethanol withdrawal (E: withdrawal 3 days). CGRP release was measured in vivo and in vitro. In vivo, CGRP release was increased in the VMH and PVN of rats following withdrawal (E: withdrawal 3 days), but not in VMH of rats following chronic ethanol (E: 0.5 g/kg in drinking water during 3 days). In vitro, CGRP release was decreased in the VMH and PVN of rats following withdrawal (E: withdrawal 3 days), but not in VMH of rats following chronic ethanol (E: 0.5 g/kg in drinking water during 3 days). These results suggest that CGRP may be involved in the adaptive response to ethanol withdrawal.

$\delta$-Adenosinomethylione ($\delta$-SAM)-dependent $\beta$-methylation of $\beta$-carboline (BC) and 1,2,3,4-tetrahydro-4-hydroxymethylisoquinoline was examined in undialyzed subfractions of guinea pig and rat brain, using HPLC/radiodetection. Simple THBCs were made from the $\delta$[2]-nitrogen by cytosolic activity possibly identical to azaheterocyclic N-methylating activity reported by others. However, simple BCs such as harmine or norharman underwent both $\delta$[2]- and 9-[indole]-N-methylations in sequential fashion by activity in the nuclear subfractions. Importantly, the 2,9-dimethyl-BC products displayed dopaminergic neurotoxic effects that surpassed the 2-methyl-BCs and approached MPP+ in the parkinsonian "street drug" toxicin. Since THBCs and BCs are endogenous constituents, their respective N-methylations of differing brain methyltransferases could lead ultimately to cationic 2,9-[indole]-methylated BC toxic factors which are trapped within the brain. Supported by MH32891 and LUNC.

COLCHICINE-INDUCED DEGENERATION OF CHOLINERGIC SEPTOHIPPOCAMPAL NEURONS AS DEMONSTRATED BY THE LOSS OF NGFR IMMUNOREACTIVITY. Sheryl R. Gine and Gary M. Provenz Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Neuronal somas in the medial septum (MS), which are immunoreactive for choline acetyltransferase (ChAT), are also immunoreactive for the nerve growth factor receptor (NGFR). A good pattern of innervation of the hippocampus by cholinergic afferents is matched by the pattern of NGFR-immunoreactive (NGFRi) fibers. Thus, NGFR-immunoreactivity appears to be a reliable marker for cholinergic somas and axons in the septohippocampal system. The present study was conducted to determine if ICV injections of colchicine, which reduce the number of ChAT-immunoreactive somas in the MS and the numbers of acetylcholinesterase (ACHE)-positive fibers in the hippocampus, produce a similar change in the numbers of NGFR-immunoreactive (NGFRi) fibers. Colchicine resulted in a 48% reduction in the number of NGFR-immunoreactive soma in the MS, compared to control. Within the hippocampus the total number of NGFRi-immunoreactive fibers were greatly reduced. These results are similar to what has been reported for ChAT-immunoreactive soma in the MS and AChE-positive fibers in the hippocampus, and support the conclusion that ICV colchicine induces degeneration of cholinergic neurons in the septohippocampal system. Supported by the Alzheimer's Disease and Related Disorders Association.


The nucleoside, 2',3'-dideoxycytidine (dDC) is a potent inhibitor of HIV but has been associated with a dose-dependent peripheral neuropathy in clinical trials. Previous studies in the rabbit have established that high doses (50-250 mg/kg/day) dDC induce a peripheral neuropathy characterized by myelin and axonal dysfunction. This severe neuropathy most likely reflected an end stage process clinically characterized by paralysis in the hindlimbs and prostration in the forelimbs. The present study was designed to establish the earlier stages and progression of dDC neuropathy. We studied 40 New Zealand white rabbits that were treated with intracisternal injections of dDC. The rabbits were divided into a control and a treated group. ddC was administered by gavage at a dose of 35 mg/kg/day. Histopathology was serially examined at 8, 12, 16, 20, 24 wks (electrophysiologic measures were recorded at these timepoints plus at baseline).

Beginning at wk 16, the proximal segments of the sciatic nerve and the associated peroneal roots showed myelin splitting and intramyelinic edema. At 20 wks, clinical signs of weakness appeared. At 24 wks, a significant reduction in axonal cross sectional diameter was evident in the sciatic and distal sciatic. At 25 wks, the sciatic was completely denervated. The sciaticopathy was stable at 24 wks in the peroneal F-Wave, which was delayed by approximately 45% of its baseline latency. These findings confirm a dDC induced proximal motor fiber myelinopathy and a distal axonopathy, and demonstrate the effectiveness of the rabbit as a model for dDC neuropathy.

SPINAL KINETICS OF LIPOSOME-ENCAPSULATED 1-B-D-ARABINO-FURANOSYL CYTOSINE IN RATS. J. Rand, S. Kim* and T. I. Yakh, Dept. of Anesthesiology and Internal Medicine*, University of California at San Diego, La Jolla, CA 92093.

Maintenance of therapeutic concentrations of antineoplastic agents in the CNS for extended periods of time is an efficient method for the delivery of the cell specific antimeatabolites in cancer chemotherapy. Multivesicular liposomes composed of bilayer lipid membrane have been reported as slow release antineoplastic drugs.

We studied the intraspinal kinetics of liposome-encapsulated (liposomal) 1-B-D-arabinofuranosyl cytosine (ara-C) in rats. Liposomes were observed in the lumbar and cervical CSF for 7 days after intrathecal (IT) injection of liposomal Ara-C (1mg/10ml) through a chronic lumbar IT catheter. The IT T1/2 of the liposomal ara-C was 154 hr and 135 hr at 7 days after IT for free ara-C. T1/2 of IT inulin-C14 in CSF was 0.5 hr. This suggests liposomes are cleared more slowly than bulk CSF flow. No toxicity on general behavior with IT, 50mg/Kg/day, ara-C, CSF sample Ara-C T1/2(1hr) R

Lumbar free 0.28 96
liposomal pellet 154 super >10 84
Cisternal liposomal pellet 58.3 9 super >10 85

R = Correlation coefficient, * = Second order kinetics.
free = unencapsulated drug, pellet = drug in liposome, super = free drug in supernatant.


577.10

Capsaicin (CAP) neonatally injected damages small primary sensory neurons related with thermo-nociceptive activity. This study analyzes the development of scratching, sniffing, grooming, rearing, sniffing, digging, and searching behaviors in intact and neonatally (day 2pp) CAP treated (50mg/Kg, s.c.) rats. The frequency of these behaviors was scored every other day from day 3 to day 90 of life. Eye opening day, body weight and physical appearance were also recorded. RESULTS: Control: Still behavior frequency (SBP) predominated over other BS's during the first 7 days of life and progressively diminished until day 13th, when rearing and digging BS's started to increase. Sniffing, grooming and searching BS's suddenly increased from day 9, however, the latter showed a fast decrement on day 13th. Scratcing BS was slightly apparent showing a small decrement. On days 14-15 eye opening occurred, CAP-treated: The frequency of all behaviors but scratching was similar as controls. Scraping BS increased significantly on day 13th and was maintained at a higher level than control throughout the study. Eye opening occurred before (days 10-14) and these rats presented home. The nociceptor stimulus (activity carried by CAP sensitive fibers) influence the time of eye opening and the intensity of scratching behavior.

SEP CH (P.P.); SEP C91, CONACyT D111-903550 (J.M.).

Bilirubin 1Xa, 4Z, 1Z (BR) depolarizes syntehpsomes, releasing acetylcholine (ACh) and ion (TPP+). The underlying mechanisms (unknown) are acutely relevant to neonatal bilirubin encephalopathy. Equilibrium distribution values were obtained for ouabain-sensitive 86Rb+ transport, and 86Rb+ influx was monitored in rat cortical synaphtosomes isolated by iso-osmotic homogenization. Purified rat synaptosomal mitochondria (35 mM) or 4 mM human serum albumin (HSA) (40 μM) BR produced a rapid (1-2 min) influx of trapped methylamine in control (Carb)-loaded synaptosomes. The rate was simulated using the weak base methylamine in two media: (a) Krebs-Ringer, (b) Ca2+- and Na+-free, 1 mM ouabain. Potassium released trapped methylamine in both conditions, but HSA/BR only showed this effect in synaphtosomal mitochondria incubated in Krebs-Ringer. Possible post-synaptic effects of BR were explored using purified T. californica acetylcholine receptor (αChR) reconstituted into Asolectin or dioleoylphosphatidylcholine-phosphatidic acid-hydrophobic base vesicles (60:20:20 mole ratio). BR (5-10 μM) did not modulate the passive or the carbamylcholine (Carb)-stimulated (200-1000 μM) 86Rb+ influx, (3) the 5-10 μM Carb-induced first-order decrease in the Carb-stimulated 86Rb+ transport, (s) the equilibration from 86Rb+-loaded pure Asolectin vesicles, (t) the equilibrium binding of 125I-α-bungarotoxin, (u) or (v) the affinity shift associated to nAChR desensitization. BR (50-100 μM) decreased the rate of binding by 20% with respect to controls, and 10 μM Penetrel shortened (3-fold) the time course of receptor desensitization to 5 μM Carb. We conclude that BR truly depolarizes the synaphtosomal plasma membrane, since intrasynaptic micromolar levels have little or no BR+ permeability, and their membrane potential is independent of K+ flux. The depolarization mechanism was investigated using a variety of BR to disrupt proton gradients, suggesting a BR-induced release of transmitter-filled vesicles known to activate the βγ subunit of the nAChR. The observed effects on rate of toxin binding and receptor desensitization may be mediated through BR-vesicular lipid matrix interactions. Supported by NIH Grants RO1 HD25341-01A2.
578.2
LACK OF EFFECT OF CHRONIC LEAD TREATMENT ON BIOAMINE METABOLITE CONCENTRATIONS IN RHUSCUS MONKEY CEREBROSPINAL FLUID (CSF). E.A. Fazioli*, R. Kraemer*, R. E. Bowman, K. J. Enge*, and D. B. Schmid. National Center for Toxicological Research, Division of Reproductive and Developmental Toxicology, Jefferson, AR; Harlow Private Laboratory, University of Wisconsin, Madison, and University School of Medicine, Nashville, TN.

Concentrations of specific metabolites (NE, DOPAC, HVA, 5-HIAA, and 3-AM) in the CSF of developing rhesus monkeys treated orally with moderate levels of lead acetate (0.05% and 0.2% of lead) did not differ from controls. Blood lead levels peaked at 62 μg/dl at 1.5 months of age and were maintained at 14 μg/dl from 20-56 months of age. Cisternal CSF samples were collected monthly from 5-41 months of age and every 1-3 months until 56 months of age. Biomimetic metabolite concentrations were assayed by high-performance liquid chromatography. As is typical of rhesus monkeys, HVA and 5-HIAA concentrations decreased with age. NE and MHPG levels remained fairly stable across time. Concentration of DOPAC began to decrease at approximately 40 months of age. However, no measure interacted with lead treatment and overall metabolite concentrations for the five measures were significantly different between control and lead-treated monkeys. As measured in cisternal CSF, biomimetic metabolites were not altered by chronic low-level lead treatment.

578.3

Low levels of lead (Pb) are proposed to damage cells at subcellular sites, including several calcium (Ca++) homeostatic pathways, mitochondria, and gap junctions. Sensitive measurements for damage at these sites were carried out with an ACAS 570 Interactive Laser Cytometer. Astroglial primary cultures were prepared from neonatal rat cerebral hemispheres. After 10-20 days in culture the cells were treated daily with medium containing 1 μg or less of Pb acetate and subcultured before examination with the laser cytometer. Intracellular calcium (Ca++) levels were measured with an Indo-1 label. In preliminary experiments astroglia responded to ionomycin by a rapid 50% increase in [Ca++]i by partial recovery towards baseline levels. Pb-treated astroglia showed a blunted Ca++ spike in response to ionomycin. In a control experiment, cells were bathed with Ca++-free medium. Astroglia not treated with Pb had no response to ionomycin. By contrast, Pb-treated astroglia showed a marked Ca++ spike followed by recovery. This finding indicates that Pb alters the handling and storage of Ca++. Other parameters measured included cell-cell communication via gap junctions, which was unaffected by Pb treatment, and mitochondrial permeability, which was reduced after one week of Pb treatment but not after three weeks.

578.4

Possibly whether perinatal lead ingestion can cause selective toxicity to central catecholaminergic nervous system in rats was tested. Three groups of Wistar rats; 1) Control, 2) Low dose and 3) High dose groups, were prepared. Right after parturition from dams rat pups received drinking water containing either 0% (control), 0.05% (low dose) or 0.2% (high dose) of lead acetate. At 2, 4, 6 and 8 weeks of age, tyrosine hydroxylase (TH) activity and Na-K ATPase activity were measured in 4 areas of rat brain. Trehalose, D-mannose, MDPB and PMA treatment for TH and ATPase activities were carried out by modified method of Reinhard et al. (1986) using H-[3,5]-L-tyrosine as substrate. TH activity was determined as a criterion of lead poisoning to central catecholaminergic nervous system and ATPase activity as a criterion of non-specific lead poisoning to any kinds of tissues. In lead exposed rats, TH activities were higher but Na-K ATPase activities were lower than those observed in age-matched control animals. Selective toxicity of lead poisoning to central catecholaminergic nervous system was evaluated by the changes of TH activities without concomitant changes of ATPase activities. Brain areas where selective toxicity of lead seems to be induced were pons-medulla (2 weeks of age) and telencephalon (6 weeks of age) in low dose group, and midbrain (4 and 6 weeks of age) in high dose group.

578.5

The study tested the hypothesis that spatial memory deficits in laboratory rats may be induced by oral administration of a daily solution containing aluminum chloride and sodium citrate. Aluminum (Al3+) is capable of binding with citrate to form a neutral Al3+ + citrate complex that may pass through internal membranes and bind to acidic subcellular sites. Long-Evans hooded rats were divided randomly into one of three experimental conditions for sixteen weeks. The control group received a daily oral aqueous solution comprised of water and sodium citrate. The low and high groups received either 100 or 1,000 ppm aluminum chloride, dissolved in a sodium citrate solution. Each animal was rotated regularly into metabolic cages for a twenty-four hour period. The animals were then behaviorally tested in the Morris water maze to assess spatial memory impairments. Brain aluminum content (BAC) was analyzed by Ethrolytic Electromagnetic Absorption Spectrophotometry. Aluminum in the urine and feces was analyzed by Flame Atomic Absorption Spectrophotometry. There was a significant difference of BAC in neuronal tissue between the high and low groups received either 100 or 1,000 ppm aluminum chloride and sodium citrate. The control group received a daily oral solution comprised of water and sodium citrate.

578.6

The presence of aluminum in the CNS has been linked to several neurological disorders, including Alzheimer's disease. AICl inhibits the release of acetylcholine catalyzed by the (Na+,K+)-ATPase of cerebral cortical synaptosomes in a dose dependent manner. At 25 μM AICl, the enzyme from adult rat brain is inhibited 14.4% and this increases to 46.8% at 100 μM AICl. Sodium citrate (100 μM), added after pre-incubation with AICl(100 μM), fails to reverse the inhibitory effect of AICl, however it can prevent inhibition of (Na+,K+)-ATPase when added simultaneously with AICl. Lineweaver Burk analysis of enzyme activity with varying concentrations of ATP indicates a reduction in the Vmax but not the Km of the (Na+,K+)-ATPase in the presence of AICl, suggestive of noncompetitive inhibition. A comparison of the enzyme in synaptosomes prepared from young or aged rats shows that the Vmax in young rats is significantly greater than that in aged rats. The susceptibility of the (Na+,K+)-ATPase to 100 μM AICl is greater in (72% inhibition) in the aged group. Disruption of the (Na+,K+)-ATPase in neurons may underlie, at least in part, the neurotoxicity associated with an elevated aluminum content in the CNS. (Supported by Alzheimer's Disease Research of the Amer. Health Assist. Found. and a gift from J. Rose.)
**578.7**

**ALUMINUM-INDUCED NEURODEGENERATION IN THE RABBIT RETINA.**


Study of the effects on the nervous system of long-term exposure to aluminum has been complicated by the unavailability of an appropriate animal model. Recent studies in this laboratory (Fry et al., Neurou. Lett. 124:216-220, 1991) have examined aluminum-induced neurofibrillary changes in rabbit retinal ganglion cells as a potential long-term toxic effect for aluminum neurotoxicity. The current study examines the dose-response and temporal relationships of the disorder. Adult New Zealand White rabbits were injected intravitreally with aluminum solutions (0.001-0.03 mg/ml) to MLW). When compared to control 54Mn-uptake by synaptosomes of mouse brain.

**578.8**

**NEUROBEHAVIORAL EFFECTS OF SUBCHRONIC DIETARY ALUMINUM IN MICE.**

M. G. Gobu, C. Keen, M.E. Gershwin. Dept Internal Medicine, Univ. California, Davis Sch. Med. Davis, CA 95616.

Female Swiss Webster mice (n=15/gp) were fed purified diets containing 25 (control) or 1000 μg Al diet (AI as Al lactate). Weight, food intake and gross neurotoxic signs were monitored regularly. At 45 and 90 days a modification of the NTP neurotoxicity screening battery was administered and spontaneous motor activity was determined using automated method for 4 24-hr period. At necropsy, brain and trace metals (Fe, Mn, Cu, Zn) concentrations were determined by AAS and lipid peroxidizability was measured by generation of thiobarbituric acid reactive substances (TBARS). Al was detected with a slight acceleration of weight gain during the second half of the 90-day period; no effect on food intake and no mortality or morbidity were observed. Rats fed 1000 μg Al diet had lowered locomotor activity (p<0.01), hindlimb grip strength (p<0.02) and air puff startle responsiveness (p<0.04) at the 90 day time point. Temperature sensitivity (tail flick test), negative geotaxis, forelimb grip strength and footsplay were not affected. Al was increased 3-fold in both brain and liver (p<0.01) but not in femur. Essential trace elements were not altered and there was no effect on TBARS measures. These results demonstrate a specific pattern of neurobehavioral deficit caused by subchronic dietary exposure (Al). We are conducting the use of further testing using more sensitive endpoints and/or longer exposure periods and higher doses. (Supported by ES49140).

**578.9**

**EFFECT OF ZnCl2 ON 54Mn-UPTAKE BY SYMNPOTOSOMES OF MOUSE BRAIN.**


The accumulation of Mn in brain decreases when given together with zinc in mice (Kendall, S. et al. Bull Environ Contam Toxicol 30: 1035-1040). We studied the effect of ZnCl2 on Mn uptake by synaptosomes. Male albino mice were killed by cervical dislocation and their brains removed immediately. Striatum (S), olfactory bulb (OB) and hypothalamus (HT) were dissected out. Synaptosomes were obtained by the technique of Hajos F.(Brain Res 93:485, 1975). Isolated synaptosomes were washed 2 times in 0.3 M sucrose and then resuspended in a 0.3 M sucrose. Samples were incubated for 0.5 min at a concentration of 10-1M. The final incubation volumes were 0.5 ml. At other assays ZnCl2 was added after a 15 min preincubation of the synaptosomes. Incubations were ended by suction through a Skatron cell harvester. The radioactivity contained in the filters were counted in a gamma counter. With a concentration of 10-5M ZnCl2 the Mn uptake was completely inhibited in OB. For S and HT a concentration of 10-6M was needed to obtain a similar inhibition. On the contrary, preincubation with the synaptosomes with 54Mn at a maximum concentration of 10-6M did not show any Mn-uptake. The inhibitory effect of ZnCl2 on Mn uptake by synaptosomes seems to be due to a higher binding affinity for ZnCl2 than Mn. The results obtained after preincubation seem to indicate that ZnMn is apparently bound in an insoluble or high affinity state to intracellular proteins.

**578.10**

**MANGANESE TOXICITY: MUSCARINIC RECEPTOR BINDING IN THE MOUSE BRAIN.**


Cholinergic neurons seem to be resistant to the toxic effect of manganese, since the activity of choline acetyltransferase was not affected in any of 5 brain regions studied in rats after eight months of treatment with manganese. We now report the effect of chronic administration of manganese on the binding of [3H]quinuclidinyl benzilate (3H-QNB) in different brain regions of mice continually treated with manganese. The results show a significant decrease in the binding of 3H-QNB to homogenates of mouse brain was performed according to the method of Yamamura and Snyder (Proc. Nat. Acad. Sci USA 75:172, 1978). The brain regions of controls and Mn-treated mice were as follows: a) striatum: 94.5 ± 0.7 and 90.8 ± 0.5; b) frontal cortex: 94.7 ± 0.5 and 93.3 ± 1.1; c) hippocampus: 92.1 ± 2.8 and 90.0 ± 3.8. The Mn-treated mice (n=4) were killed by cervical dislocation two days after the end of the intoxication. Brains were extracted immediately and the following regions dissected out: striatum, hippocampus and frontal cortex. The binding of 3H-QNB to homogenates of mouse brain was performed according to the method of Yamamura and Snyder (Proc. Nat. Acad. Sci USA 75:172, 1978). The brain regions of controls and Mn-treated mice were as follows: a) striatum: 94.5 ± 0.7 and 90.8 ± 0.5; b) frontal cortex: 94.7 ± 0.5 and 93.3 ± 1.1; c) hippocampus: 92.1 ± 2.8 and 90.0 ± 3.8. The Mn-treated mice (n=4) were killed by cervical dislocation two days after the end of the intoxication. Brains were extracted immediately and the following regions dissected out: striatum, hippocampus and frontal cortex. The binding of 3H-QNB to homogenates of mouse brain was performed according to the method of Yamamura and Snyder (Proc. Nat. Acad. Sci USA 75:172, 1978). The brain regions of controls and Mn-treated mice were as follows: a) striatum: 94.5 ± 0.7 and 90.8 ± 0.5; b) frontal cortex: 94.7 ± 0.5 and 93.3 ± 1.1; c) hippocampus: 92.1 ± 2.8 and 90.0 ± 3.8. The results are presented in the present study clearly show that chronic manganese administration does not alter the binding constants of 3H-QNB to the muscarinic cholinergic receptors in mouse brain.

**578.11**

**AN IMMUNOCHEMICAL STUDY OF THE EFFECTS OF TRIMETHYLPENII ON PEPTIDIC NEURONS WITHIN THE HIPPOCAMPUS.**


It is well established that trimethyltin (TMT) causes loss of projection neurons in the CA1 and dentate/granule cell of the temporal region of the dentate gyrus. The effects of TMT on hippocampal neuron containing peptides and synaptic transmission has been less well documented. Immunocytochemistry visualized vasoactive intestinal polypeptide (VIP), somatostatin (SS), and calcitonin gene-related peptide (CGRP) in the hippocampus of male Long-Evans rats exposed to 6 mg/kg TMT. When compared to control cells counts revealed a significant loss of neurons showing VIP- and SS-like immunoreactivity in rats exposed to TMT. CGR appeared to be less affected by the toxin. These effects resemble those produced by cerebral ischemia (Bingaman et al., Acta Neuropathol. 73:110-114, 1987) and may be interpreted to indicate that loss of SS neurons which possess excitable inhibitory GABAergic neurons, may contribute to loss of CA1 pyramids after TMT. (Supported by NIH Grant ES-04705-05 to LLM)

**578.12**

**TRIMETHYLTIN (TMT) AND GLUTAMINE-EVOKED RELEASE OF GLUTAMATE FROM HIPPOCAMPAL AND CAUDATE SLICES OF RAT BRAIN.**


We have previously reported that although the hippocampal neurons progressively increase the levels of hippocampal glutamine in both 7 and 17 month old rats, it produces passive avoidance deficits in hippocampal neuronal necrosis and gliosis only at 17 months. Glutamate levels in hippocampal extracts were reduced in the older rats, while their glutamate receptors (KA subtype) were increased. Although TMT had little effect on total glutamate content (in vivo), there might be a selective effect on extracellular release. Therefore, we superfused glutamate and TMT hippocampal and caudate slices and measured glutamate in the effluent (LM) produced by a dose-related increase in glutamate release of at least 15-fold, while addition of TMT (up to 13 μM) only slightly increased this release. Since potassium increased the glutamate effect, but in a non-concentration-related fashion, we are at present uncertain of the exact mechanism of the glutamate effect. However, our results are consistent with the possibility of an indirect action of TMT to produce neurotoxicity secondary to glutamine-evoked release of glutamate.
578.13  

Routes of entry and distribution of metals into the CNS have toxicologic significance. To determine the distribution of bismuth (Bi), adult female Sprague-Dawley rats were injected i.p. with either 200 or 250 mg/kg Bi in substrate (2-ethylhexanoate) at 100 ppm in ethanol to establish a Bi depot. Four weeks later, mice were anesthetized and perfused with a saline flush. Brain and spinal cord were processed for frozen and paraffin sections. Bi was visualized with silver staining and mice brain sections with Alizarin blue. Control sections had negligible Bi. In dosed mice, sonata of brainstem (2-3 ppm) was relatively high levels of Bi were also found in lumbosacral spinal cord and ventral/dorsal gradient in cerebellum. These patterns suggest mechanisms associated with neuronal sonata. White matter had few grains except for regions of crossover neurons close to the subependymal organ. Choroid plexus had strong grains and aggregates. There were several gradients of Bi, including a ventral/dorsal gradient in cerebellum. These patterns suggest mechanism for entry and distribution of Bi into CNS that may apply to other metals.

578.14  
REGIONAL BISMUTH LEVELS AFTER PARENTERAL DOSING IN MICE AND FERRITS.  J.F.  Ross and G.T.  Lawhorn*.  Miami Valley Laboratories, Procter & Gamble Co., Box 398707, Cincinnati, OH 45239-8707.

Bismuth (Bi) neurotoxicity (encephalopathy) occurs rarely during long term oral treatment with Bi-containing medications. Signs of neurotoxicity (hyporeflexia, myoclonus, ataxia) were observed in these animals. In previous studies, we have shown that mice are produced by injection of Bi (Bross et al., NeuroToxicology 9(4):381-8, 1988). Here we measured regional brain Bi levels of animals and different substrates from different animals. Bi and the sodium salt of Bi sublimate (HNO3) or DEO (s-4+) was injected IP into mice (BiHNO3, BiNO3 or BiP), Ferrat. Parts of CNS and PNS were dissected and Bi was measured by atomic absorption spectrometry/hydride generation after wet ashing with acid. BiNO3 (250 mg/kg) was injected once and regions analyzed 24 days later (mice: n=9) or (100 mg/kg) twice to four times during eight weeks (ferrets: n=11). BiNO3 was dosed (20 mg/kg/day) to mice (n=10) five/week for 12 weeks. Bi levels in ferrets to the hypothalamic Bi level and compared by analysis of variance followed by Student's t-test or a rank sum test adjusted for multiple comparisons. Bi distribution was independent of type of Bi dosed, the dosing regime or the species tested. In the CNS, levels were highest in meninges (15 ppm). Relatively high levels of Bi were also found in lumbar spinal cord (20 ppm), hypothalamus (4 ppm), olfactory bulb (4-7 ppm) and brainstem (2-4 ppm). Significantly lower levels were found in cerebellum, striatum and cerebral cortex (1-2 ppm). The data complement subclinical studies in the nervous system, and concentrations in sites consistent with the signs of neurotoxicity.

578.15  

Methyl mercury (MeHg) increases the release of neurotransmitters and intracellular calcium ([Ca2+]i) in mammalian neuron terminals. The Ca2+ pool(s) responsible for the elevated [Ca2+]i and transmitter release are unknown. Less is known about the effects of organic mercury (Hg) on nerve terminal [Ca2+]i and transmitter release. Ruthenium red (RR), a polysaccharide dye, decreases MeHg-induced acetylcholine release from synaptosomes and [35S]Ca2+ efflux from synaptosomes in vitro. We examined the ability of RR to prevent elevations in synaptic [Ca2+]i by MeHg and HgCl2 in nerve terminals of the Syrian hamster. In fura-2 loaded synaptosomes, MeHg and HgCl2 (10 μM each) increased [Ca2+]i by 55 and 121 nM respectively in media containing 200 μM MeHg. Pretreatment with 10 μM HgCl2 prior to Mn2+ addition decreased the 340/380 ratio. This effect was altered by pretreatment with 20 μM MnCl2. This indicates that Bi is inhomogeneously distributed in the nervous system, and concentrations in sites consistent with the signs of neurotoxicity.

578.16  
ALUMINUM TREATMENT IMPAIRS THE ABILITY OF ASTROGLIA TO PROTECT MUCONEURONS AND CORTICAL NEURONS FROM GLUTAMATE EXCITOTOXICITY.  L.B.  Sant and B.H.  Aschkin.  Dept. of Anatomy, University of Saskatchewan, Saskatoon, Canada, S7N 0W0.

Aluminum has been implicated as a neurotoxin in a number of neurological disorders, including Amyotrophic Lateral Sclerosis, Alzheimer's disease, and senile dementia. Glutamate may, under pathological conditions, act as an excitotoxin. In experiments designed to examine synaptic interactions between aluminum (Al) and glutamate, we have demonstrated that aluminum treatment of astrocytes impaired their ability to protect neurons from glutamate excitotoxicity. Astroglial cells were isolated from newborn CDI mice and cultured on coverslips for 2 weeks. Mice were isolated from E15 CD1 mouse embryos and cultured on coverslips for six days. Astroglial and neuronal cultures were then treated with aluminum chloride at a final concentration of 100 μM for 3 days. Control or Al-treated astrocytes were co-cultured with the control or Al-treated motoneurons; these co-cultures were treated with glutamate at a final concentration of 50 μM. Cell viability was determined 24 hours later using the fluorescein diacetate-propidium iodide fluorescence technique (Jones and Senft. 1985. J Histochem Cytochem, 33:77). Glutamate did not affect the viability of control or Al-treated neurons when co-cultured with control astrocytes, however, glutamate caused death of all neurons (both control and Al-treated) when neonates were co-cultured with Al-treated astrocytes. These experiments have been repeated using cortical neurones and similar results. Our observations demonstrate that sublethal levels of aluminum will severely impair the ability of astroglial cells to protect neurones from glutamate excitotoxicity, rendering normally subtoxic levels of glutamate lethal to neurons. This suggests that impaired astroglial function may play a causal role in diseases involving neuronal degeneration. We are currently comparing glutamate uptake in control and Al-treated astrocytes.

578.17  

Membrane damage related to lipid peroxidation has been implicated in the pathophysiology of a variety of diseases. The Upjohn Company has developed a novel compound, known as the lazeroids, aimed at preventing the propagation of the lipid peroxide reaction chain. Several of these compounds have been tested in an in vitro model of Fe2+-induced lipid peroxidation. Iron-labeled neuronal cultures were treated with various lazeroids at concentrations ranging from 0.3 to 100 μM. The cultures were then exposed to 200 μM ferrous ammonium sulfate for 30 min to induce lipid peroxidation. Membrane damage and cell viability were determined by the fluorescent probe, 2',7'-dichlorofluorescin diacetate, and by the fluorescent probe, 3,3'-diaminonaphthalene-2,7-disulfonic acid. This assay provides a neuronal culture method of detecting compounds that protect against Fe2+-induced lipid peroxidation and subsequent membrane dysfunction and death.
578.19
CEREBELLAR GRANULAR CELL MODELS FOR TESTING OF PROTECTION AGAINST NEUROTOXICITY. G.J. Picq*, J.S. Althaus, and P.F. von Voigtlander. The Upjohn Company, Kalamazoo, MI 49001

A novel in vitro method for testing various lipid peroxidation inhibitors (lazaroids) ability to protect cerebellar granular cells has been developed. These neurones possess excitotoxic receptors and have been used in studies involving excitotoxic amino acids. We have postulated that they would be susceptible to injury induced by bathione sulfite (BSS), an inhibitor of γ-glutamylcysteine synthetase and ferrous ammonium sulfate (PAS), an inhibitor of lipid peroxidation. This study reveals BSS (10-100μg/mL) after 24 h, produces a dose-dependent reduction of glutathione (GSH) in the intact cells, as determined by HPLC with electrochemical detection, along with a reduction of cell viability as measured by amino-iso-butyric acid (AIB) uptake. Data will also be presented which shows the importance of glutathione in FAS toxicity. In addition, our results show that pretreatment with lazaroid can block these toxic insults. It is hoped that the described toxicity models will reveal mechanisms of lazaroid-mediated neuronal protection.

578.20
LIPID HYDROPEROXIDES IN CEREBRUM AND CEREBELLUM OF RAT BRAINS. Eli Kaplan. Research Service, VA Medical Center, Minneapolis, MN 55417

As a prelude to studies on the metabolism of arachidonic acid in the brains leading to the formation of lipid hydroperoxides, studies were done to determine if rapidly frozen tissues could be used as a control. Male, 30-35 g, rats, 150-200 g, were fasted overnight prior to perfusion with 5% heparin in K-R bicarbonate buffer, pH 7.6. Cerebrum and cerebellum were quickly excised, dropped into liquid N2, and kept at -70°C for 5 days. In the 2nd set of experiments the brain tissues were quickly excised, homogenized in same buffer and lipids extracted using Bligh-Dyer's method. The frozen tissue was homogenized and lipids extracted in a similar manner. The lipid classes were separated by SPE, and individual lipid hydroperoxides identified and quantitated by reverse-phase HPLC. Results suggested that all the HPETEs in the frozen group was 3 times greater than in the freshly homogenized cerebrum and cerebellum. 12- and 9-HPETEs were found in the largest quantity in both groups. The quantity of HPETEs in the cerebellum appeared to be greater than the cerebrum. It is apparent that tissues, frozen in liquid N2, produce large quantities of HPETEs, and can not be used as a control.

579.1

Hydrogen sulfide (HS) is suspected to cause asphyxiation and death by an action on respiratory neurons in the brainstem. Dorsal raphe (DR) nucleus allows the pump to reactivate, and 4) inhibits synaptic transmission. Hydrogen sulfide's effects on respiration may be caused by any or all of these actions.

579.2

Polychlorinated biphenyl (PCB) is a common pollutant, the ingestion of which is harmful to a number of physiological systems in adult animals. Previous studies on our in vivo model have found addition of 250ppm (PCB 126), to the maternal diet during pregnancy and lactation to depress both thyroid status and choline acetyltransferase (CHAT) activity in the hippocampus and basal forebrain of 15 day old rats. The present study was done to determine whether the maternal ingestion of PCB results in a proportionally lesser depression of thyroid status and Chat activity. Thyroid status was determined by measuring circulating T3 and T4 levels with RIA. Chat activity was estimated quantitatively by the ability of a homogenate to incorporate [3H]-acetyl-CoA into acetycholine and qualitatively by immunohistochemistry. All PCB doses significantly depressed thryoxine (T4) levels but had insignificant effects on triiodothyronine (T3). Quantitation of Chat activity revealed a linear dose response with less Chat activity in the hippocampus and basal forebrain as the PCB doses increased. Fewer neuronal processes of PCB fed animals stained positively for Chat than in controls. Thus, there seems to be a significant physiological influence of maternal PCB ingestion at doses lower than 250 ppm (125, 62.5 ppm).
ACRYLAMIDE ALTERS THE DISTRIBUTION OF MAP1 AND MAP2 IMMUNOREACTIVITY IN RAT BRAIN. N.B. Chaudhuri*, M.I. Sabri, P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201.

Microtubule-associated proteins (MAPs) play an important role in the formation and maturation of neurites. Two MAPs (kinetin and dynein) mediate microtubule-based fast axonal transport which is dependent on progressive spatiotemporally characterized by neurotoxic degradation in New Zealand white rabbit. Strong M.J. et al., Lancet, 330:640, 1990; Strong M.J. et al., Acta Neurologica, 81:235, 1991). Using conventional assays for cell viability (trypan blue dye exclusion, LDH, MTI) and cell proliferation (H-thymidine incorporation, immunostaining), we studied the effects of NBBS on C6 glioma cells and primary astrocytic cell lines, respectively. Micromolar (μM) concentrations of NBBS inhibited cell growth and produced morphologic changes and cell death. A lower concentration of NBBS was required to inhibit DNA synthesis in Neuro-2a cells (10 μM) than in C6 cells (100 μM), suggesting that neuronal cells are more sensitive than glial cells. Thymidine incorporation was reduced in confluent, actively growing C6 glioma cells was affected more than confluent cell monolayers. The effect of NBBS on DNA synthesis in C6 cells occurred as early as 24 h; a concentration of 10 μM (18% inhibition of thymidine incorporation), with 30% inhibition at 72 h. At an NBBS concentration of 250 μM, DNA synthesis was strikingly inhibited to about 100 μM and 250 μM NBBS exhibited markedly reduced or absent immunoreactivity with antibodies against S-100 protein and glial fibrillary acidic protein. Our data indicate a cell type-specific neurotoxicity of NBBS and support our earlier studies in rabbits. Studies are underway to determine the effects of NBBS on primary cultures of neuronal cells.

EFFECTS OF PERINATAL EXPOSURE TO SPECIFIC POLYCHLORINATED BIPHENYL (PCB) CONGENERS ON LOCOMOTOR ACTIVITY OF RATS. S.I. Schantz and D.K. Ness*. Institute for Environmental Studies and Dept of Veterinary Biosciences, Univ of Illinois at Urbana-Champaign, Urbana, IL 61801.

Individual PCB congeners vary greatly in toxic potency. Those with 0 or 1 ortho chlorines, 2 para chlorines and at least 2 meta chlorines can assume a coplanar configuration similar to TCDD and are extremely toxic. Those with 2 or more ortho chlorines cannot assume a coplanar configuration and are believed to be considerably less toxic. However, recent evidence suggests the structure-activity relationship may be different for neurotoxicity. That is, congener-inhibited neurotoxic than coplanar PCBs. Perinatal exposure to low-doses of complex PCB mixtures has been shown to alter activity in rodents, monkeys and humans. The first experiment examined acute effects of maternal exposure of rats exposed to complex PCB mixture in rat pups. The second experiment used: triadimefon an agricultural triazine herbicide that induces "stimulant-like" effects when administered to rodents. Previous reports from this laboratory indicated that low doses of riemannin caused acute motor effects. At higher doses, more intensively stereotypic behaviors (e.g., head weaving and backward locomotion). We have also reported that triadimefon administration, generally decreases dopamine concentrations in striatum, and increases them in olfactory tubercles. The fungicide was found not to have direct effects on D1 or D2 dopamine receptors. These initial investigations led to the hypothesis that triadimefon acts like an indirect-acting dopamine agonist, either inhibiting dopamine uptake or inducing dopamine release. The present study was designed to test these hypotheses. The first experiment examined whether triadimefon would inhibit the uptake of dopamine, serotonin, or norepinephrine. This assay was run in crude synaptic fractions and prepared and measured uptake of each radiolabeled neurotransmitter. Using striatal synaptosomes, triadimefon dose-dependently inhibited the uptake of [3H]-dopamine with an IC50 = 29 μM, about one-fifth the potency of GBR 12909, a selective blocker of dopamine uptake. Triadimenol, a mammalian metabolite of triadimefon and a commercial triazole fungicide, was 2-3 fold less potent than triadimefon in its ability to inhibit the uptake of [3H]-dopamine. Triadimenol did not alter the uptake of either [3H]-serotonin or [3H]-noradrenergine into synaptosomes prepared from frontal cortex (IC50 > 100 μM). The second experiment used slices of rat striatum to assess the ability of triadimefon to release dopamine. Amphetamine (1 μM), used as a positive control, doubled the basal efflux of preloaded [3H]-dopamine. Conversely, 31 μM triadimefon had no effect on release. Together, these data suggest that triadimefon is a relatively selective inhibitor of dopamine uptake with potency sufficient to explain its behavioral and neuromodulatory effects. (Supported in part by ES01104 and ES07126)
EFFECTS OF EXPOSURE TO TRIPHENYL PHOSPHITE (TPP) ON THE CNS OF THE CHICK (GALLUS DOMESTICUS) D. Tanaka, Jr., S.J. Bursian and E. Lehning, Depts. of Anatomy and Animal Science and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824.

Although injections of young rats to PTN and the resulting inhibition of ACHE produced a dose-dependent and varied between 6-30%. Growth was inhibited only during the agonist binding properties were unaffected by PTN. Thus, prolonged exposure of young rats to PTN and the resulting inhibition of ACHE produced a decrease in locomotion but did not influence the binding of acetylcholine to the receptor. Supported by US EPA.

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579.15

STIMULATION SITE AT OR NEAR THE BEND.

579.16

3-NITROP Propionic ACID - EXCITOTOXIC LESION OF THE HIPPOCAMPUS INDUCED BY AN INHIBITOR OF CHEMICAL ENERGY METABOLISM. R. Blumenkranz, L. A. A. Andrade, R. A. R. B. A. Spencer, C. N. Allen. Center for Research on Occupational and Environmental Toxicology, Oregon Health Science University, Portland, Oregon 97201

3-Nitropropionic acid (3-NPA), a widespread fungal and plant toxin, and inhibitor of mitochondrial Complex II is of pathogenetic significance in human and animal diseases. The pattern of damage in the basal ganglia and the hippocampus in vivo mimics excitotoxic lesions. In mouse cortex explant cultures, 3-NPA induces vacuolization and chromatolysis, which are preceded by cellular energy depletion and partly attenuated by glutamate antagonists (MK-801).

579.17


Several studies suggest that neurite initiation and elongation may be modulated by intracellular free calcium ion concentrations and/or calcium fluxes across the plasma membrane. Departures from optimal intracellular calcium concentrations, both increases and decreases, appear to lead to decreased neurite initiation (I. Frank abstract, this meeting), as does blocking calcium influx through voltage-sensitive calcium channels (Ausdeirk et al., 1990). The organochlorine pesticide lindane has been reported to increase intracellular calcium concentrations in short-term experiments (less than an hour). We cultured embryonic rat hippocampal neurons and mouse N1E-115 neuroblastoma cells in lindane concentrations ranging from 0.1 to 100 uM. Neurite initiation and elongation were measured after 48 hours in culture. Lindane significantly inhibited neurite initiation at concentrations as low as 1 uM (hippocampal neurons) and 0.5 uM (N1E-115 cells). Lindane significantly inhibited neurite elongation at concentrations as low as 25 uM in both cell types. Contrary to previous reports using short-term exposure, digital imaging microscopy with fura-2 showed that long-term exposure to lindane (24 hours or more) decreased intracellular free calcium ion concentrations in a dose-dependent manner.

CLINICAL CNS NEUROPHYSIOLOGY

580.1


A 9-11 cm segment of amphibian nerve was placed in a relatively large flat-bottomed plastic trough containing Ringer's solution. The distal end of the nerve emerged from the solution through a monopolar recording. A 3 x 10 cm figure 8 magnetic coil (MC) was positioned beneath the trough, with the long axis of the monitoring centered under and parallel to the nerve. The waveform of the energizing pulses (polyphasic, phase II 80% of phase I, biphasic, phase II 20% or 35% of phase I) was selectively changed by a mechanical switch.

Suprathereshold biphasic pulses elicited a single response corresponding to the (-) going spatial derivative of the electric field, just distal to the anterior diverging nerve roots. Reversing the current shifted the latency by 0.65-1.0 ms, corresponding to approximately 30 mm at the measured conduction velocity. This second site at the figure 8 is posterior to its middle region. These locations were confirmed by direct electrical stimulation. For the two current directions, the responses were stable in latency at all stimulus output intensities.

Polyphasic MC pulses of either polarity resulted in stimulation at the spatial derivative site closest to the recording electrode; the responses differed in latency by approximately 200 usec possibly reflecting the period between 1st and 2nd phases. Presumably, excitation by the 1st or reversed polarity 2nd phase of the polyphasic pulse at this site leads to collision with impulses generated at the more distant site.

These findings may help explain MC excitation of axons in the CNS.
NONINVASIVE MEASUREMENT OF NERVE ELECTRICAL MEMBRANE PROPERTIES IN HUMANS USING VARIABLE WIDTH MAGNETIC STIMULATORS. D. Rudyak, E. Marx. Sch. of Optometry, U. Calif., Berkeley, 94720.

Using strength-duration protocols, magnetic stimulators with one to seven electrodes, and transcranial stimulation, the basic membrane properties were measured. The threshold stimulus for the stimulation of the motor cortex was found to be 380 μT for a 2 ms pulse. For the measurement of the compound muscle action potential, the threshold stimulus was 100 μT for a 1 ms pulse. The results of these measurements are consistent with the findings of other investigators.

TEMPERATURE CHANGES IN DEPTH ELECTRODE DURING MR SCANNING. L. Zhang, C. Wilson, M. Levkovsky, L. Behnke and R. Lutfi. Brain Research Institute and Department of Neurosurgery and Department of Radiology, UCLA, Los Angeles, CA 90024.

Surgical therapy for complex partial epilepsy requires precise identification of the seizure focus. Magnetic resonance imaging (MRI) compatible depth electrodes have been implanted stereotaxically in the temporal lobes for prolonged stereoelectrocorticography (SEEG) recording to assist in focus identification. Post-implantation MRI scans offer accurate localization of electrode sites; however, eddy currents induced by the radiofrequency magnetic field applied by the MRI scanner may heat the electrode. Electrode heating may raise the local brain tissue temperature, possibly to a dangerous extent. We therefore examined electrode temperature changes in a 1.5-T MRI scanner.

A bundle of five Nickel-Chromium electrodes (NiCr, Nickel 80%, Chromium 20%) were attached around a miniaturized precision anchor. The last 25 mm of each wire is bared, so that 50 mm of electrode is exposed. Implantation is done with a jacketed translumbar aortogram needle under fluoroscopy control. 5 electrodes implanted in psoas, gluteus and paraspinal muscles were monitored.

RESPONSE OF ESSENTIAL WRIST TREMOR TO SINUSOIDAL SOMATOSENSORY FEEDBACK. R. J. Fife and C. A. Higgins*. Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794-9230.

Sinusoidal forcings at 4 to 15 Hz were delivered by a computer-controlled torque motor to the wrists of 11 normal adults and 10 patients with advanced essential tremor. Wrist angle and forearm EMG were recorded. Our normal adults exhibited a second-order underdamped wrist response with a natural frequency of 5.5 Hz. The wrist response of our patients exhibited 68% greater resonance at forcing frequencies of 4 to 6 Hz, which is the frequency range of their tremor. The increased resonance in our patients was attributable to intermittent frequency entrainment of essential tremor by the mechanically-induced somatosensory feedback. The wrist response of our patients and controls did not differ at frequencies greater than 7 Hz. The lack of entrainment of motor units by essential tremor precluded normal reflex modulation of forearm EMG by wrist forcings at frequencies greater than 6 Hz. Consequently, the forearm EMG response of our patients was less than control values at frequencies greater than 6 Hz. Furthermore, forcings at frequencies greater than 12 Hz tended to suppress essential tremor. Essential tremor is a centrally generated oscillation that can be enhanced or suppressed by somatosensory feedback. (Supported by NIH HD grant HD20793.)


In order to understand the biomechanical aspects of gait in DS, we have analyzed the muscle activations and movement patterns during free gait of 12 young subjects of different age with DS as well as of 10 normal subjects. Gait movements have been monitored by an electrogoniometer and surface EMG was recorded from four leg muscles (hamstrings, quadriceps, tibia alis ant., triceps s.). Three patterns of muscle activations have been identified: the first one showed EMG character similar to that observed in the normals, the second pattern was characterized by the precocious activation of triceps surae and was observed in most DS subjects, the third pattern was mainly represented by the activation of the triceps surae at the beginning (0-35%) and at the end (70-100%) of the step cycle. Our results show the presence of a different phenotypical expression of walking patterns in DS and of a specific delay in the functional development of the triceps surae. Moreover they suggest a key role for this muscle and its neural control in the delayed development of gait observed in this syndrome.


Thalamotomy has long been used in the treatment of intractable tremor. The optimal site for the lesion appears to be in the region immediately anterior to the thalamic ventrobasal complex where cells respond to joint movement and/or fire in synchrony with the tremor. However, it is unclear whether these criteria predict the optimal lesion site and also whether stimulation-induced tremor reduction (TR) is due to disruption and canalization of neural activity or to activation of neural elements. The present study utilized microinjections of the local anesthetic lidocaine hydrochloride (1-2 ul of 1% solution in 1% lidocaine) into the ventralis intermedius (Vim) and ventralis oralis (Vop) nuclei. Injection sites were confirmed by histology and are designated Vip and Vop, respectively.

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Sinusoidal forcings at 4 to 15 Hz were delivered by a computer-controlled torque motor to the wrists of 11 normal adults and 10 patients with advanced essential tremor. Wrist angle and forearm EMG were recorded. Our normal adults exhibited a second-order underdamped wrist response with a natural frequency of 5.5 Hz. The wrist response of our patients exhibited 68% greater resonance at forcing frequencies of 4 to 6 Hz, which is the frequency range of their tremor. The increased resonance in our patients was attributable to intermittent frequency entrainment of essential tremor by the mechanically-induced somatosensory feedback. The wrist response of our patients and controls did not differ at frequencies greater than 7 Hz. Consequently, the forearm EMG response of our patients was less than control values at frequencies greater than 6 Hz. Furthermore, forcings at frequencies greater than 12 Hz tended to suppress essential tremor. Essential tremor is a centrally generated oscillation that can be enhanced or suppressed by somatosensory feedback. (Supported by NIHHD grant HD20793.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991 THURSDAY PM


There are two major impediments to the widespread use of functional electrical stimulation to improve motor function in paralyzed patients: i) unreliability of electrodes; ii) unreliability of sensors and controllers. We have tested a robust intramuscular electrode similar to that developed recently in Cleveland by J.T. Mortimer. Two Cooner 632 insulated stainless steel wires were inserted through a needle into the triceps surae. The wires were circularly insulated by a 400 μm polyurethane tubing and terminated with a knotted monofilament barb which acts as an anchor. The last 25 mm of each wire is bared, so that 50 mm of wire is available for entry through the skin. Muscle implantation is done with a jacketed transulnar aortogram needle under fluoroscopy control. 5 electrodes implanted in psoas, gluteus medius muscles and pectoral nerve in 2 incomplete quadriplegics have remained electrically stable and functional for a year. We have also developed a portable microprocessor controller which uses signals from underfoot force sensors to control the stimulation during gait. The system compensates for transducer drift and can be monitored and parametrically adjusted from an IBM AT host. Supported by Canadian MRC.

Funded, in part, by the Minerva Foundation, Berkeley, California.

The optimal site for the lesion is believed to be in the region immediately anterior to the thalamic ventrobasal complex where cells respond to joint movement and/or fire in synchrony with the tremor (tremor cells) and where microstimulation blocks or reduces the tremor. However, it is unclear whether these criteria predict the optimal lesion site and also whether stimulation-induced tremor reduction (TR) is due to disruption and canalization of neural activity or to activation of neural elements. The present study utilized microinjections of the local anesthetic lidocaine hydrochloride (1-2 ul of 1% solution in 1% lidocaine) into the ventralis intermedius (Vim) and ventralis oralis (Vop) nuclei. Injection sites were confirmed by histology and are designated Vip and Vop, respectively.

In order to explore the basic mechanisms underlying the disturbance and delayed motor development in DS, we have analyzed the muscle activations and movement patterns during free gait of 12 young subjects of different age with DS as well as of 10 normal subjects. Gait movements have been monitored by an electrogoniometer and surface EMG was recorded from four leg muscles (hamstrings, quadriceps, tibialis ant., triceps s.). Three patterns of muscle activation have been identified: the first one showed EMG character similar to that observed in the normals, the second pattern was characterized by the precocious activation of triceps surae and was observed in most DS subjects, the third pattern was mainly represented by the activation of the triceps surae at the beginning (0-35%) and at the end (70-100%) of the step cycle. Our results show the presence of a different phenotypical expression of walking patterns in DS and of a specific delay in the functional development of the triceps surae. Moreover they suggest a key role for this muscle and its neural control in the delayed development of gait observed in this syndrome.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991

580.0
ABNORMAL BLOOD FLOW RESPONSES TO VIBRATION IN PATIENTS WITH WRITER'S CRAMP. I.W. Tempel, J.S. Perlmuter. Departments of Neurology and Radiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We evaluated sensorimotor processing in patients with writer’s cramp using position emission tomography (PET) and H2O. We studied 6 right-handed patients (3 female) 24-72 yrs of age (mean 50). All had unilateral writer’s cramp. There were 4 male patients (1 female) 20-72 yrs of age (mean 41). Data were analyzed using subtraction image analysis. Vibrations produced a consistently localized and robust peak response (peak height vs. %ID in primary sensorimotor cortex (SI) contralateral to hand vibration in normals (right hemisphere: 13.11 +/- 3.25; left hemisphere: 12.57 +/- 1.18). This response was significantly reduced in patients (p < .001) whether vibrating the unaffected (58 +/- .62) or affected (10.40 +/- 2.00) hand. Coordinates for the SMA locus were determined from the averaged responses in an independent data set of 14 normals 20-69 yrs of age (mean 59). It was in high medial frontal cortex contralateral to the vibrated hand. This locus then was used to objectively search the data from the patients and the other 8 normals. Right and left SMA responses in normals were not significantly different from each other (right hemisphere: 6.52 +/- 1.47; left hemisphere: 6.46 +/- 1.45). There also was no significant right-left (or "unaffected-affected") difference in cramp patients (unaffected hemisphere: 4.30 +/- 1.70; affected hemisphere: 4.66 +/- 1.09). Both SMA responses were significantly less than in normals (p < .05). These data indicate that patients with unilateral writer’s cramp have bilateral brain dysfunction. Abnormal responses in SI and SMA are consistent with dysfunction of the cortico-subcortical-thalamo-cortical circuit.

580.11

In previous studies of temporal lobe epilepsy (TLE) patients, we have shown that hippocampal theta activity recorded from vertex trajectory depth electrodes changes as a function of behavior. In this study of nine patients with TLE, theta activity recorded from an occipital trajectory hippocampal depth electrode (contralateral to the seizure focus) was compared across four behavioral conditions: resting eyes closed (RC), resting eyes open (RO), eyes open with auditory word stimulation (AW), eyes open with visuospatial activation (AP). Theta activity decreased significantly during AW and AP compared to RC (p < .0002). The differences between AP and AW were significant (p < .001). In contrast, vertex trajectory patients showed a significant decrease in theta during RO compared to AW, and no significant differences between AW & RC or AP & RO. The differences between the vertex and occipital groups in behavior-related theta activity changes may be due to: group differences, small size of the occipital group, or an electro-physiologically important difference in electrode placement. Occipital trajectory electrodes allow comparison between contact points along a line from the amygdala through anterior hippocampus to posterior hippocampus. It is interesting to note that the significant behavior-related changes in theta activity were observed in the amygdala, and that theta changes within the hippocampus varied from anterior to posterior suggesting that precise electrode placement is an important consideration.

580.13

The pathogenetic mechanisms underlying brain dysfunction in Tay-Sachs disease and other neuronal storage disorders are poorly understood. Two abnormalities of neurons known to commonly occur in neuronal storage disorders are (i) ectopic dendritogenesis and associated asymmetrical synapse formation on axon hillocks of pyramidal neurons, and (ii) axonal spheroids, which result in disorganization of the neuronal cytoskeleton. We have evaluated the relative contributions of these cytopathologic features to cortical dysfunction using feline models of GM1 gangliosidosis (GM1) and Niemann-Pick disease type C (NPD). To date we have studied 1 GM1 cat (9 mo old), 1 NPD cat (7 mo old), and 4 normal cats (9 mo and older). Laminar profiles of field potentials, current source density, and multiunit activity, elicited by 90 dB, 100 sec, contralateral clicks, were sampled from primary auditory cortex under pentobarbital anesthesia. In the GM1 cat, response latencies in laminae III-IV were elevated by 2-7 ms and response durations by 4-10 ms over normal values, whereas in the NPD cat elevations were minimal. In both diseases the amplitude of the early paraventricular response was increased. In the GM1 cat, response latencies were 56% greater than normal at rates above 10 Hz; 100% attenuation was reached at 40 Hz, vs. 200 Hz for normals. Control data suggested a minimal contribution from abnormal pathologic and electrophysiologic features. While there is clear evidence for neurophysiologic abnormalities in the cerebral cortex of both GM1 and NPD cats, the relative contributions of increased excitatory and decreased inhibitory influences remain to be elucidated.

580.14

Neural electromagnetic measurements offer the prospect of high resolution, noninvasive mapping of dynamic patterns of activity of the human brain. However, localization of neural sources from external magnetic field or potential measurements requires the solution of an ill-posed inverse problem. We have developed an approach based on MUSIC (Multiple Signal Classification) algorithms that systematically exploits spatio-temporal correlations in the measurement matrix. The method also incorporates anatomical information, derived from volumetric magnetic resonance imaging, to constrain the locations of candidate sources. The signal subspace of the electromagnetic measurements is estimated by performing an eigenvalue decomposition on the correlation matrix of the time series data. Because theoretical and experimental evidence suggests that the dominant sources observed in noninvasive electromagnetic measurements are cortical dendritic currents, in our application the source region is limited to cortex. A scan metric is computed at each spatial location in the source region by projecting the field of a current element at that location onto the signal subspace, to identify sources that produce fields that lie within the signal subspace. Locations that produce a peak in the 3-D distribution of the metric are accepted as probable localization candidates. In this manner, the source region can be broken down to the timecourse of component sources is required, sources identified by the algorithm may be used as an estimate for multiple dipole fitting procedures. We have applied these procedures to experimental neuroanatomic data from somatosensory studies to resolve the sources activated by electrical stimulation of the thumb, ring finger or the simultaneous stimulation of both. We have also resolved neuroanatomic sources associated with early cortical activation in response to a small sinusoidal grating presented to single quadrants of the visual field. Our MUSIC-based algorithm provides a powerful and systematic approach to estimate a spatio-temporal ensemble of sources, while incorporating anatomic information to improve the accuracy and efficiency of electromagnetic imaging of brain function.

580.10
THURSDAY PM  CLINICAL CNS NEUROPHYSIOLOGY

580.10

Alterations in central serotonergic neurotransmission have been implicated in the pathophysiology of major depression. We studied central serotonergic (5HT) function in depression by comparing the neuroendocrine response to pharmacologic challenge with the point SHT releaser agent fenfluramine (FEN) both between depressed (n=21) and healthy (n=17) subjects and within a depressed subset (n=12) later treated with desipramine (DMI). Serum cortisol (COR), prolactin (PRL), and growth hormone (HGH) were measured at baseline, and at 30 mins. and 2 and 5 hrs after an oral dose of FEN (60 mg). Results showed a significantly (p<0.05) increased (± SEM) peak change in COR in depressed (6.61 ± .91 μg/dl) as compared to healthy (0.64 ± 1.62 μg/dl) subjects. This enhanced COR response to FEN was significantly attenuated and appeared to normalize following DMI treatment (0.94 ± 1.42 vs 33.7 ± 85 μg/dl). No significant differences in PRL or HGH responses were noted. These findings lend support to the hypothesis of altered SHT function in depression which normalizes with antidepressant treatment.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991

SYMPOSIUM: CORTICAL MECHANISMS

585.1 SPONTANEOUS CALCIUM TRANSIENTS IN DEVELOPING NEOCORTICAL NEURONS

L. C. Katz and R. Yuste. Dept. of Neurobiology, Duke University, Durham, NC

Spontaneous activity in the developing visual system plays a critical role in the activity-dependent formation of circuitry. Since synaptic activation causes postsynaptic calcium influx, video-based optical recording of single neuron [Ca2+]i can be used to monitor activity over extensive cortical regions, with single cell resolution. Using fura-2, we have now recorded spontaneous [Ca2+]i changes in slices of somatosensory and visual cortex of neonatal kittens during the first postnatal week. Most of the events were observed in the visual cortex, as distinguished by their spiny dendrites and large cell bodies. These events are non-periodic and occur in complex spatial and temporal patterns. Individual transients last from 1 to 10 min.

Supported by the Lucille P. Markey Charitable Trust.

585.2 NEURONAL GROUPS REVEALED BY OPTICAL RECORDING OF CALCIUM TRANSIENTS IN SLICES OF DEVELOPING NEOCortex

R. Yuste, A. Baranyi and L. C. Katz. Lab. of Neurobiology, Rockefeller University, NY, USA 10021 and Dept. of Neurobiology, Duke University, Durham, NC 27710.

Intracellular recording techniques were used to study the physiological properties, visual evoked potentials and spatiotemporal spread of visually evoked synaptic activity in 28 visually responsive area 17 neurons recorded in 6-day-old kittens, with 70 μM glass micropipettes filled with K+–methylene blue dye. Stability of recordings was monitored with minimal dye delivery (1–2 mm), polarized light microscopy to study the physiological properties, visually evoked potentials underlying functional selectivity, and oscillatory behavior of visual cortical neurons. In upper layers, groups of co-active cells tile the cortex without significant overlap. Groups are present at birth, and remain similar in size and properties throughout the first postnatal week. To explore possible relationships between co-active groups and patterns of thalamic innervation, we imaged Ca2+ imaging with fluorescent anterograde labelling of the "barrel field" in slices of somatosensory cortex. Rhodamin was injected in the thalamic nuclei and the resulting pattern of "barrels" imaged in a tangential slice through layer 4. In preliminary experiments, groups, which are smaller than individual barrels, occur both inside and outside the barrels without any clear relationship to them. In numerous cases, groups were observed at identical locations several minutes apart. This, combined with the lack of overlap with neighbouring groups, suggests that they may represent modular units in the developing brain.

Supported by the Lucille P. Markey Charitable Trust.

585.3 INTRACELLULAR STUDY OF VISUAL RESPONSE PROPERTIES AND OSCILLATORY BEHAVIOR OF KITTEN AREA 17 NEURONS AT THE PEAK OF THE CRITICAL PERIOD


Intracellular recording techniques were used to study the physiological properties, visually evoked potentials and spatiotemporal spread of visually evoked synaptic activity in 28 visually responsive area 17 neurons recorded in 6-day-old kittens, with 70 μM glass micropipettes filled with K+–methylene blue dye. Stability of recordings was monitored with minimal dye delivery (1–2 mm), bilateral thalamo-neocortical and extrathalamic connections.

1) The membrane potential (-63 ± 7 mV), spike amplitude (54 ± 11 mV), and membrane conductance of the 77 cells recorded were 70 ± 10 mS, with 70 μM glass micropipettes filled with K+–methylene blue dye. The resting membrane potential of 50-100 μm in diameter. These synchronously active cells elicits a clear mutual temporal relationship to one another. Groups occur in both tangential and coronal slices, in visual and somatosensory cortices, and in all cortical layers, being more frequent in the upper layers. In tangential slices, groups of co-active cells tile the cortex without significant overlap. Groups are present at birth, and remain similar in size and properties throughout the first postnatal week.

To explore possible relationships between co-active groups and patterns of thalamic innervation, we imaged Ca2+ imaging with fluorescent anterograde labelling of the "barrel field" in slices of somatosensory cortex. Rhodamin was injected in the thalamic nucleus and the resulting pattern of "barrels" imaged in a tangential slice through layer 4. In preliminary experiments, groups, which are smaller than individual barrels, occur both inside and outside the barrels without any clear relationship to them. In numerous cases, groups were observed at identical locations several minutes apart. This, combined with the lack of overlap with neighbouring groups, suggests that they may represent modular units in the developing brain.

Supported by the Lucille P. Markey Charitable Trust.

585.4 POSTSYNTAPTIC MEMBRANE POTENTIAL REGULATES POTENTIATION AND DEPRESSION OF VISUALLY EVOKED SYNAPTIC POTENTIALS IN KITTEN CORTICAL NEURONS RECORDED IN VIVO


It has been shown in vivo extracellular recordings in cat visual cortex that changes in coherence between pre- and postsynaptic activity are correlated with post-synaptic membrane potential changes in spiny stellate and pyramidal cells. This work was supported by HSF grant to F.Y. and CNRS Fellowship to A.B.
ORIENTATION AND DIRECTION SENSITIVE CELLS IN THE LGNd AND STRIATE CORTEX OF DARK REARED CATS. Kirk G. Thompson, Yifeng Zhou, Steven J. Ault Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

LGNd and striate cortical (area 17) cells of cat reared in darkness from birth were studied quantitatively to assess the effects of monocular deprivation (MD) during the critical period of mammalian visual cortex development. The ocular dominance (OD) distribution in area 17 was similar to that of the non-deprived eye. This effect is ascribed to competition between the thalamic afferents. We tested the hypothesis that this competition is for a neurotrophic factor such as NGF by assessing whether an exogenous supply of NGF could prevent the effect.

Most LGNd cells and cortical cells were orientation selective when the test stimuli were gratings rather than random noise. All grating cells in the LGNd exhibited a phase-dependent enhancement of orientation sensitivity coupled with early times less variable than those of cortical cells. Also, in both the LGNd and in area 17, cells were more selective when the test stimuli were gratings rather than random noise. Area 17 cells did not differ quantitatively or qualitatively. The orientation sensitivity of LGNd relay cells and area 17 cells did not differ quantitatively or qualitatively.

Direction sensitivity of LGNd relay cells and area 17 cells was also assessed. LGNd cells were directionally selective when tested with low and high spatial frequency gratings, whereas area 17 cells were not. The percentage of direction selective cortical cells in dark reared cats was 58.5% of recorded neurons in area 17. 57.1±12.1% of recorded neurons in area 17 were binocularly responsive compared to 9.6±0.6% and 6.3±1.7% in kittens treated with cytochrome c (n=3; 172 cells) or untreated (n=3; 148 cells), respectively. Receptive field properties of neurons in NGF-treated kittens did not show any abnormalities. Auto-radiographic experiments utilizing 125I-NGF are in progress to detect the possible presence of NGF binding sites in visual system-related nuclei. A specific role of NGF on visual cortical plasticity is proposed.
585.1 REDUCED TYROSINE HYDROXYLASE IMMUNE-REACTIVITY IN FOCUS CORRELATES OF SUICIDE VICTIMS A. Byerley, L. Lawrence Berkeley Laboratory, USA. Part of a search for presynaptic noradrenergic markers in suicide and depression, we have investigated the fate of noradrenaline synthesising enzymes in suicide victims. Antibodies against tyrosine hydroxylase (TH), the rate limiting enzyme in noradrenaline synthesis, and against the beta hydroxylase (DBH) of the last enzyme in the synthesis, were used for immunohistochemical staining of human brain locus coeruleus sections obtained postmortem. Brains of 6 suicide victims and 10 normal controls were examined. Beta- and sex matched controls were obtained from the medical examiners’ office. All subjects were drug- and neuropathology free at time of death and had no psychiatric history (controls). TH levels were significantly lower in the suicides (controls: 20.8±6.6 fmol/mg protein; suicides: 14.4±4.5 fmol/mg protein; p<0.005). These findings represent the first study in which both [3H]PAR and [3H]-IMI were used in these experiments. Scatchard analysis of these data were performed. The diagnostic groups studied were: (1) young suicides, n=14, (<50 yrs); (2) elderly normal controls, n=14 (>60 yrs); (3) elderly suicide victims, n=14 (>60 yrs). TH levels were significantly reduced in the elderly suicide victims, but increased in the elderly normal controls. All cases were labelled with fluorescent tracers and ocular dominance (OD) maps described by the projection of the visual field of each eye upon the contralateral superior colliculus. In 3 additional mature animals a similar rotation was performed after anaesthesia immediately prior to mapping. In all of the early rotation group the projection of the visual field was distorted towards a complicated map in which elevation was in accord with eye rotation but azimuth was represented in the original orientation on the colliculus. This may indicate some developmental compensation in the colliculus towards restoring spatially correct vision from the misaligned eye.

585.2 BINDING TO SEROTONIN UPTAKE SITES IN PREFRONTAL AND TEMPORAL CORTEX OF SUICIDE VICTIMS B.A. Hennetf, V. Arango*, and J.J. Mann. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

We have previously reported increased postsynaptic 5-HT, binding in the prefrontal cortex of suicide victims (Arch. Gen. Psychiatry, 43 596-599, 1986; Arch. Gen. Psychiatry, 47 1038-47, 1990). Conflicting reports exist in the literature as to whether binding to the serotonin transporter on serotonin nerve terminals is reduced in brain cortical regions of suicide victims. Using the very selective ligand, 5-HT1A, and platelets of patients with major depression. However, not all investigators have confirmed the reduction in [3H]-imipramine [3H]-IMI binding site density in drug-free depressed patients. This may be at least partly due to the fact that [3H]-IMI is not the ideal ligand to label the presynaptic 5HT transporter as it binds to a variety of other neurotransmitter receptors. For all of these reasons we have used a very selective 5HT uptake inhibitor, [3H]-paroxetine ([3H]-PAR), to label the 5HT transporter to study the platelet binding kinetics of the 5HT transporter in drug-free depressed patients and age- and sex-matched controls. In the same platelet samples, we also measured [3H]-IMI binding. Seven concentrations of [3H]-IMI and [3H]-PAR were used in these experiments. Scatchard analysis of these data were performed. The diagnostic groups studied were: (1) young normals, n=14, (<50 yrs); (2) elderly normal controls, n=14 (>60 yrs); (3) young major depression, n=14 (<50 yrs); (4) elderly major depression, n=14 (>60 yrs). The number (Bmax) of high affinity binding sites for both [3H]-PAR and [3H]-IMI was significantly reduced in the depressed patients when compared to their age-matched controls. No change in Kd was observed. In addition, there was a significant correlation between the Bmax for [3H]-PAR and [3H]-IMI (p < 0.05). These findings represent the first study in which both [3H]-PAR and [3H]-IMI binding was measured in identical platelet samples. The results support a role for SHT systems in the pathophysiology of depression. (Supported by NIMH MH- 40169.)
586.5

Department of Psychiatry, The Medical College of Pennsylvania, Philadelphia, PA 19129, USA.

The sensitivity of the traditional spontaneous alternation paradigm to serotonergic manipulation has been previously demonstrated. The present study used a modified version of the spontaneous alternation task, as an improved model for the repetitive nature and inescapability seen in obsessive-compulsive disorder (OCD) in humans. Food-deprived rats were run in a black Plexiglas maze with identical arms, equipped with white end-panels. All arms were baited with a small amount of chocolate milk. Each rat was placed in the center and allowed to explore the maze until 12 arm entries were completed (or a maximum of 10 min). Chocolate milk was replenished after each choice. The percent of repeated arm entry patterns was recorded as well as the latency to complete 12 entries. After a stable baseline of arm entries was achieved the effects of manipulating the serotonergic system were tested.

Both the nonselective 5-HT agonist 5-MeODMT (1.25 mg/kg) and the more selective 5-HT₁A agonist 8-OH-DPAT (0.2 mg/kg) disrupted alternation patterns in the 4-arm maze, a change that was reversed by the nonselective antagonist methysergide (0.1 mg/kg). A course of chronic treatment (2 × 5 mg/kg for 21 days) with the selective 5-HT uptake blocking agent fluoxetine had a protective effect on the 5-MeODMT-induced disruption of alternation behavior, whereas chronic treatment with the benzodiazepine clorazapaxepin did not.

586.6

THE PHOSPHOLIPID THEORY OF DEPRESSION: IMPLICATIONS FOR TRH FUNCTION IN CNS. J. L. Hibelhöf* and A. Sattin. DVA Sepulveda-UCLA Medical Center, Sepulveda, CA 91434-2099.

Many confirmed biological findings in depressed patients not explained by amine theories are compatible with increased activities of phospholipase A₂ (PLA₂) (Hibelhöf et al., Biol. Psychiat., 25: 945, 1989). By altering membrane phospholipid composition and fluidity diverse enzymes and regulatory proteins may be affected. The theory explains depression-related altered activity of Na-K-ATPase, β₂- and α₂-adrenergic receptors, MAO, uptake of NE and 5-HT, and imipramine binding. PLA₂ reduction of tyrosine and tryptophan hydroxylase activity is consistent with amine theories. The theory explains the antidepressant effects of trycyclic antidepressants, carbamazepine (CBZ), and some other antianxiety agents. PLA₂ activation may reduce the binding of TRH and cortisol to their respective ligands, explaining the reduced responses to TRH and dexamethasone seen in many depressed patients. ECS activates phospholipase C-α-arachidonic acid-PEGE (prolonged elevation), an anticonvulsant which inhibits PLA₂. Reduced PLA₂ activity might increase TRH receptor efficacy in CNS (as in pituitary) wherein TRH synthesis and release are also augmented, in hippocampus, after ECS (Kobolch et al., Abstr. Soc. Neurosci., p: 1998, 1990). This original concept augmented of TRH function might be opposed by receptor down-regulation but the functional resultant remains unknown. Both ECS and TRH are anticonvulsants. These interactions are believed to contribute to the uniquely antidepressant properties of Electroconvulsive Treatment which is also anticonvulsant in humans (suppt: DVA Res. Scv.)

586.7

ELEVATED CEREBRAL CORTEX Gₛ-ALPHA IMMUNOREACTIVITY IN BIPOLAR AFFECTIVE DISORDER. L.T. Young, P.P. Li, J.J. Krish, K.P. Sus* and J.J. Wash. Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

Evidence implicating postreceptor disturbances in affective disorders, including bipolar affective disorder (BAD), together with the recent observations that lithium acts on the coupling of neurotransmitters to effector responses at the guanine nucleotide binding (G)-protein level suggest that abnormalities in G-protein function may occur in BAD. To test the hypothesis that G-protein levels are altered in brains of BAD patients we estimated the relative abundance of G-protein subunits in postmortem brain obtained from patients (N=7) who had been diagnosed as BAD (confirmed by chart review) and age and sex-matched controls (N=7). G-protein subunit immunoreactivities were determined in membranes prepared from postmortem prefrontal cortex (Broadman's area 10) using SDS-PAGE and immunoblotting with specific polyclonal antibodies against specific G-protein subunits: Gₛ, G₁α, G₂α, and G₃α. Immunoreactive bands were detected by enhanced chemiluminescence and quantitated by densitometry. Brain samples from BAD and matched control subjects were processed in a yoked design on the same blot to control for autoradiographic differences, with cortical Gₛ subunit (52 kDa species) immunoreactivity being significantly elevated (34%, p<0.01) in BAD subjects compared with age and sex-matched controls. In contrast, no significant differences were found in the other G-protein subunits measured. On the basis that increased Gₛα immunoreactivity reflects increased Gₛ α subunit concentration, and that increased levels may enhance functional responses to receptor activation, the present findings suggest that disturbances in Gₛ-mediated function may occur in BAD. To test the hypothesis that G-protein levels are altered in brains of BAD subjects compared with age and sex-matched controls. In contrast, no significant differences were found in the other G-protein subunits measured.

586.8


Blocking GABA neurotransmission in the region of the DMH of rats elicits a constellation of responses which include increases in heart rate (HR), respiratory rate (RR), blood pressure (BP), locomotor agitation, averse responses and "anxiety" similar to severe anxiety states like panic attacks in humans. The present study was aimed at testing if this response can be blocked by treatment with standard human anti-panic drugs like imipramine. Rats were then randomized to either daily placebo injections or imipramine (15 mg/kg) injections intraperitoneally for 7 days. All treatments were double-blind and the physiological responses to BMI injection (25 mg/250 nl) into the DMH were recorded at baseline and on days 2, 7 and 10. The BMI response was almost completely blocked by systemic imipramine while the placebo treatment had no significant effect. These results support the hypothesis that GABA blockade in the DMH may be a model for severe anxiety states. (Supported by R29 MH45362-02.)
flickery single channels having a unitary conductance of ~26 pS and an antagonist of ATP-dependent K+ channels, produced a depression of the current in the absence of cromakalim. Exposure of the cells to energy-depleting conditions (oligomycin 0.24 μM) caused a prolonged burst duration (mean open time ~ 131 ms); similar channel openings were observed in the presence of external Ca2+. Several agents that raise the temperature was raised from 22° to 30°C and the single-channel conductance increased. This voltage-dependent K+ channel was inhibited by a rise in internal Ca2+ concentration produced by mitochondria or lonomycin in the presence of external Ca2+. Several agents that raise cyclic AMP levels in T lymphocytes caused an increase in K+ channel activity in cell-attached patches (8-Br-CAMP, dbcAMP, cpt CAMP, forskolin, isoproterenol, isobutyl methyl xanthine, histamine and prostaglandin E3). The cAMP-analogs had no effect on intracellular Ca2+ as measured by Fluo-3 fluorescence. Supported by grants from MRC and NCIC (Canada).

587.3 Hormonal Regulation of K+ Channels in Human T Lymphocytes. L.C. Schlichter*, P.A. Pahapill* and P.S. Pennefather, Department of Physiology, University of Toronto, Ontario, Canada. M55 1A8.

In cell-attached patch-clamp recordings of intact human T cells we found voltage-activated K+ channels in about 60% of patches. The I-V relation was inwardly rectifying and had a slope conductance of 9.4 pS to 23.8 pS. From the reversal potentials under various ionic gradients about 0 mV when the bath contained 150 mM KC1 saline. Ensemble currents constructed from single channel recordings showed sigmoidal kinetics of current activation and a monoexponential decay phase. These kinetics were well fitted by a Hodgkin-Huxley type n4 j kinetic model with time constants very similar to the whole-cell current of disrupted cells. K+ channel activity at the resting potential increased about 10 fold when the temperature was raised from 22° to 30°C and the single-channel conductance increased. This voltage-dependent K+ channel was inhibited by a rise in internal Ca2+ concentration produced by mitochondria or lonomycin in the presence of external Ca2+. Several agents that raise cyclic AMP levels in T lymphocytes caused an increase in K+ channel activity in cell-attached patches (8-Br-CAMP, dbcAMP, cpt CAMP, forskolin, isoproterenol, isobutyl methyl xanthine, histamine and prostaglandin E3). The cAMP-analogs had no effect on intracellular Ca2+, as measured by Fluo-3 fluorescence. Supported by grants from MRC and NCIC (Canada).

587.4 STIMULATION OF ATP-SENSITIVE K+ CHANNEL ACTIVITY BY ADP IN EXCISED PATCHES FROM CULTURED MOUSE PANCREATIC B-CELLS. W.E. Hopkins, F.R. Ribero1, D.L. Cook*, Dept. of Physiology/Biophysics and Medicine, Univ. of Washington and VA Medical Center, Seattle, WA 98108.

ATP and ADP are known to compete in regulating ATP-sensitive potassium channels (K(ATP)) in insulin-secreting cells, yet little is known about the receptor mechanisms for this effect. We have obtained concentration-response data for ATP on K(ATP) channel activity, as has been described (Cook and Hall, 1994). Furthermore, we found that 10 mM ATP inhibited channel activity by 94 ± 3% (sem, N=7 patches) suggesting that it is a full rather than partial agonist for inhibition of K(ATP) channel activity. At millimolar concentrations, ADP inhibited K(ATP) channel activity by 19 ± 7% (N=6). The stimulatory effect of ADP did not run-down with repeated applications, and was not mimicked by the non-hydrolyzable ADP analogue, ADP-β-S (100 μM). In 20% of patches in which K(ATP) channels were blockable by 10 mM ADP, the excitatory effect of 100 μM ADP was not seen. These results suggest that ADP competes with ATP by binding to an ADP site which opens the channel while ATP (and higher concentrations of ADP) binds to a separate site which closes the channel. This competition may be the basis for channel regulation by ADP levels in the 10-100 μM range thought to exist in cells.
587.7 THE DIAVENTAL CATION BINDING SITE ON POTASSIUM CHANNELS. T. Brodaczek and S. Sieber, Department of Pharmacology, University of Rochester, Rochester, NY 14622. 

External divalent cations produce significant alterations in the kinetics of delayed rectifier potassium channels of squid (Loligo pealei) axon. The activation of the K channel current is slowed and the rate of deactivation is increased. These effects are produced by cations physiologically important in Ca2+. However, the kinetic alterations are more pronounced when Zn ions are used as probes. The modifications produced by low concentrations (2 to 5 mM) of Zn ions are qualitatively similar to but much larger than those produced by much larger concentrations of Ca ions. At normal pH values (7.5), these Zn concentrations produce a 3- to 4-fold slowing of the activation time constant: much too large to be consistent with any reasonable voltage shift produced by interaction with surface charges. The K+ for hydrogen ion inhibition of the Zn effects is near 7.4. Treatment with hydrogen peroxide or the hydroxyl-modifying reagent does not alter the Zn effects, however, Zn ions have almost no effect on the K channel kinetics after treatment with the amino-specific reagent trinitrobenzenesulfonil fluoride (TNBS). The external divalent cation binding site on the squid delayed rectifier K channel probably contains one or more important amino residues: lysine or a terminal amine. 

To see if these properties of the divalent cation binding site are characteristics general to K channels we performed experiments on a K channel with a known sequence: the A-type K channel from Shaker Drosophila. The baculovirus vector expression system was used to insert A-type K channels from Shaker into the cell membranes of caterpillar cells (Spodoptera frugiperda). The caterpillar cells have no endogenous ion channels. Electrophysiological studies were performed using standard voltage-clamp techniques. The interaction of Zn with the divalent cation binding site of the Shaker A-type channel produces some effects similar to those seen in squid: the activation and inactivation kinetics are slowed by Zn; and hydrogen ions compete with Zn for the binding site. However, there is a major difference: treatment of the cell with TNBS does not render the Zn inactive.

587.9 ANALYSIS OF CURRENT-VOLTAGE RELATIONS OF THE DELAYED RECTIFIER CHANNEL (Iq) BY THE SINGLE VACANCY MODEL OF ION TRANSLATION. J.R. Clay, NIH, Bethesda, MD 20892. 

The Iq channel in squid axon is known to be multiply occupied by 2 or 3 K ions, based on tracer flux measurements (Beggstech and DeWeer, 1980; JGP 76:83). The other measure of ion permeation is the net flux, i.e., the current-voltage (IV) relation, which for Iq is well described by the Goldman-Hodgkin-Katz flux equation (Clay, 1984; Biophy J 45:481). Inferences about multiple occupancy cannot be drawn from the net flux. Nevertheless, the IV and tracer flux results taken together are suggestive of a paradox inasmuch as the GHK equation is based on independent movement of ions across the membrane. The paradox can be resolved by the single vacancy model of ion translation in which the channel is either fully saturated or has, at most, a single vacancy (Rohler and Heckmann, 1979; JTB 79:381; Schumaker and MacKinnon, 1990; Biophy J 58:979). A 3-burst site model of the model in the low tonic strength (physiological) regime gives IV relations for Iq which are essentially equivalent to GHK for -200 < V < 200 mV. The model is also consistent with the tracer flux results. In other words, the resolution to the paradox is that the Iq channel may indeed obey the independence principle for ion vacancies rather than ion occupancies.

587.10 Characterization of the biosynthesis, processing and assembly of the α1K channel α-peptide expressed in Xenopus oocytes and mammalian cells. J.S. Trimmer and A. Kleinschek, Depts. of Pharmacol. and Obstet. and Gynecol., Univ. of Rochester, Rochester, NY 14642. 

The biochemical properties of the α1K channel α-peptide expressed from the cloned cDNA (Frech et al., Nature 340:642, 1989) in transient expression systems have been studied. Using two distinct anti-α1K sera, we have identified the α1K α-peptide in Xenopus oocytes microinjected with α1K cDNA that express large, voltage-sensitive K+ currents identical to those previously reported. This α1K α-peptide is a broad band centered at an Mr of 118 kD; the endogenous α1K α-peptide in adult rat brain has an Mr of 130 kD. The α1K cDNA sequence predicts a core peptide of 95.3 kD, the α1K α-peptide in both Xenopus oocytes and rat brain has undergone extensive posttranslational modification. To investigate these processes in mammalian cells, the α1K α-peptide was expressed in a human embryonic kidney cell line and was found to have an Mr = 113 kD. Pulse chase studies in these cells showed a conversion of the initial Mr = 95 kD product to the final Mr = 113 kD product. These results show that post-translation processing makes a significant contribution to the final structure of the α1K α-peptide in rat brain, Xenopus oocytes and cell lines.


Whole cell patch clamp techniques were used to record K+ currents from myometrial cells isolated from immature (23-25 days postpartum) rat uterus and human myometum (fibroid) tissue. IRB approval has been given. 

Immaturing rat myometrial cells responded to depolarizing voltage steps from holding potentials of -800 mV to +200 mV with fast outward currents that decayed with two time constants (6.8±7.69 (sem) msec and 75.77±15.92 (sem) msec). When the animals are injected prior to cell isolation with 17β-estradiol, the probability of expression of these currents is reduced from 78.6% (n=42) in control cells to 29.8% (n=47) in cells from estrogen-injected animals. Cells from progestosterone-injected animals showed little difference from control. 

Fast transient outward currents have also been recorded predominantly in human myometromus cells (55.9%, n=23 in myomatous cells compared to 5.9%, n=34 in normal cells). Human myomatous cells, therefore, may reflect undifferentiated myometrial cells.

587.12 XENOPUS MYOCYTES DIFFERENTIATING IN VITRO EXPRESS SEVERAL CLASSES OF TRANSIENT POTASSIUM CHANNELS. L. Ernberg and R.C. Sieber, Department of Biology, UCSD, La Jolla, CA 92030. 

Currents through voltage-dependent potassium channels affect the amplitude and duration of action potentials and consequently regulate events which are secondary to membrane depolarization. Thus, developmental changes of potassium currents play an essential role in the differentiation of excitable cells. We are investigating the development of transient potassium currents in Xenopus myocytes differentiating in vitro. Single channel recordings are performed in the cell-attached and inside-out configuration of the patch clamp technique with myocytes grown in the presence of different differentiating cell types, in particular sympathetic neurons. 

Channels were identified by their steady-state inactivation, which was complete at 40 mV in the inside-out configuration. Upon depolarization from a negative holding potential, the channels were activated, showing faster activation with more depolarized potentials. Inactivation during depolarizing voltage steps was indicated by the decay of ensemble average currents. In some patches the current decay could be best fitted with 2 exponentials, in others with one. Time constants of current decay were variable, but clustered around 10 and 300 ms in the inside-out as well as the cell-attached configuration. However, in excised patches, channels giving rise to rapidly decaying currents were observed less often than in cell-attached patches. These results indicate the presence of several classes of transient potassium channels. 

The density of channels in cell-attached patches of myocyte surface membrane increased during the first day in vitro. However, at all times some arrays of ensemble average currents were similar for channels at both developmental stages. The lack of change of kinetic properties suggests that an increase in the density of potassium channels rather than the expression of new classes of channels contributes to the developmental increase in density of transient potassium currents. Supported by MDA (UE) & NS25916 (NCS).

588.1 ACTIVATION OF DIFFERENT PROTEIN KINASE C ISOENZYMES IN IMMORTALIZED LHRRH-NEURONS. W.C. Wetsel*, H. Rivera*, and I. Merchenthaler. Lab of Molecular and Integrative Neuroscience, NIEHS, Res. Tri. PK, NC 27709.

An immortalized, hypothalamic, neuronal cell line (GT-1-7) has recently been developed which synthesizes and secretes LHRRH (Melton et al., Neuron 5, 1990). In vitro, LHRRH nerve terminals in the median eminence are highly regulated by synaptic inputs and their release involves specific phosphotyrosine residues. The 12-13 s half-time of these processes is much shorter than the 1-2 min half-time of PLP secretory events. These results suggest that LHRRH synthesis and release involve distinct mechanisms. In contrast, the 2 s half-time of PLP secretion involves an entirely different mechanism.


Previously (Neurosci Abst, 28:1143), we discussed the merits of mathematical models of vasopressin (AVP) synthesis based exclusively on one of three possible mechanisms (transcription, translation, or mRNA decay). Our results suggested that a strictly transcriptional model best fits the data. In this report, we have expanded our model to include both transcriptional and translational mechanisms.

588.3 NEURAL PATHWAYS INVOLVED IN OSMOTICALLY-STIMULATED THIRST AND VASOPRESSIN SECRETION. A. Dohanics, G. E. Hoffman, and J. G. Verbalis. Depts. of Medicine, Physiology, and Psychiatry, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

In order to help delineate the neural circuitry subserving osmotically-stimulated thirst and vasopressin (VP) secretion, we have utilized levels of C fos protein to identify neurons activated following IV infusion of hypertonic solutions. These studies have been combined with the retrograde labelling of neurons projecting to the supraoptic nucleus - a site responsible for the production of VP. Twenty three Sprague Dawley rats were used and on day 1 all had femoral veins cannulated. Eight of these also had stereotaxically placed, bilateral injections of a mixture of rhodamine-labelled paraformaldehyde. Elevated levels of C fos protein were found in neurons of the brain that were subsequently found to be responsive to osmotic stimuli. In normonatremic/sham-operated rats strong VP-NP staining was present only in the paraventricular and supraoptic nuclei. These areas were essentially free of immune-reactive neurons in control rats. In double labelling experiments, at least 20% of fos reactive neurons in the SFO, MnPO, and OVLT projected to the supraoptic nucleus. These results can be summarized as follows: the population of neurons in the forebrain which are responsive to osmotic stimuli recovers beyond baseline levels; both predictions are observed experimentally. This modification also explains the unexpectedly slow VP recovery under antidiuretic conditions (which blocks the age-dependent synthesis). Finally, assuming that transcriptional mechanisms regulate both stimulated and age-dependent VP synthesis, the excess VP release following CCK administration can be prevented by inducing hypophagia.

By incorporating constant, age-dependent VP synthesis into the transcriptional model (mRNA half-life=9 days), we have successfully accounted for three quantitative shortcomings of the original model. This modification also explains the rate of osmotic AVP and AVP release after chronic stimulation and explains why CCK responses occur at baseline levels; both predictions are observed experimentally. These studies have been combined with the retrograde labeling of neurons projecting to the supraoptic nucleus - a site responsible for the production of VP. Twenty three Sprague Dawley rats were used and on day 1 all had femoral veins cannulated. Eight of these also had stereotaxically placed, bilateral injections of a mixture of rhodamine-labelled paraformaldehyde. Elevated levels of C fos protein were found in neurons of the brain that were subsequently found to be responsive to osmotic stimuli. In normonatremic/sham-operated rats strong VP-NP staining was present only in the paraventricular and supraoptic nuclei. These areas were essentially free of immune-reactive neurons in control rats. In double labelling experiments, at least 20% of fos reactive neurons in the SFO, MnPO, and OVLT projected to the supraoptic nucleus. These results can be summarized as follows: the population of neurons in the forebrain which are responsive to osmotic stimuli recovers beyond baseline levels; both predictions are observed experimentally. This modification also explains the unexpectedly slow VP recovery under antidiuretic conditions (which blocks the age-dependent synthesis). Finally, assuming that transcriptional mechanisms regulate both stimulated and age-dependent VP synthesis, the excess VP release following CCK administration can be prevented by inducing hypophagia.

588.4 CHRONIC HYPONATREMIA PREVENTS SPRIUTING OF OXYTOCINIC AXONS IN LONG-TERM TRANSCONTINENT NUCLEUS (CTN) DEFICIENT RATS. J. Dohanics, G. E. Hoffman, and J. G. Verbalis. Dept. of Medicine and Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

DeSTRUCTION of the VP canistate anxious rat's hypothalamic nuclei by stereotaxic injection of 30nl of rhodamine-labelled paraformaldehyde prevented significant anxiolytic-like effects in the open field test. The VP canistate anxious rat's hypothalamic nuclei by stereotaxic injection of 30nl of rhodamine-labelled paraformaldehyde prevented significant anxiolytic-like effects in the open field test. The VP canistate anxious rat's hypothalamic nuclei by stereotaxic injection of 30nl of rhodamine-labelled paraformaldehyde prevented significant anxiolytic-like effects in the open field test. The VP canistate anxious rat's hypothalamic nuclei by stereotaxic injection of 30nl of rhodamine-labelled paraformaldehyde prevented significant anxiolytic-like effects in the open field test. The VP canistate anxious rat's hypothalamic nuclei by stereotaxic injection of 30nl of rhodamine-labelled paraformaldehyde prevented significant anxiolytic-like effects in the open field test. 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588.7


Objective: To assess oxyrhynchus regulation of water balance in normals (N) and polydipsics with schizophrenia (PDS) and without (PNS) hypotonic hyponatremia.

Methods: PDS (n = 12) received two 2% NaCl infusions (0.1 ml/kg:min), 35° post-infusion each received either 10 ml/kg of water or nothing. Plasma vasopressin (pAVP), desire for water (DFW), and relevant stimuli were measured at 0, 10, 30, 45, 55, 60, 65, 70, and 75 °post-infusion. Matched PHSs (N) and PNSs (N) only ingested water post-infusion.

Results: pAVP dropped immediately in Ns following water (0° = 4.8 ± 9 pg/ml, 40° = 2.6 ± 7) vs. nothing (0° = 5.1 ± 1.8, 40° = 5.1 ± 2.3) prior to changes in stimuli. pAVP also fell immediately in both PHSs (0° = 7.2 ± 8, 40° = 7.3 ± 3) and PNSs (0° = 3.1 ± 1.9, 40° = 1.6 ± 1.2), and their responses resembled Ns. In contrast, desire for water dropped acutely in Ns (0° = 2.6 cups, 40° = 3.2) and remained level; while PNSs had basal DFW (5.6 ± 1.7) which fell acutely (40° = 18 ± 2.1) but rapidly rebounded (55° = 5.4 ± 2.9). PHSs (30°/45°) showed no change over time.

Conclusions: Defects in oxyrhynchus regulation of water balance are unlikely to account for our previously described deficits in AVP regulation in PDS; but may contribute to the relatively difficulty of polydipsia in PHSs and PDSs. Supported by Scottish Rite Foundation (MBIG), R29 MH43618 (MBI), GCRC M 01R0055.

588.9

EFFECTS OF DIETARY PROTEIN INTAKE AND AMINO ACIDS ON TSH SECRETION. H. Edelhoch*, J. Gubler, Dept. of Internal Medicine-Endocrinology, University of Mainz, 6500 Mainz and Dept. of Physiological Psychology, University of Trier, 5500 Trier, FRG.

In previous studies we have investigated the effects of meals and precursor amino acids on prolactin and cortisol secretion. In the present study, we examined the effects of dietary protein and amino acids on basal and stimulated TSH secretion in normal volunteers. TSH levels were determined in seven different experimental situations: administration of placebo, tyrsoine (5 g), l-tyrosine (10 g), l-tyrosine (10 g) given with 2.6 cups water, and l-tyrosine (10 g) given with 2.6 cups water and 15 g of glycine. In all experimental situations, TSH levels did not significantly change except in the last one where TSH levels significantly increased (p<0.05).

588.10

REGULATION OF DBI Peptide and Mitochondrial BENZODIAZEPINE RECEPTOR (MBR) EXPRESSION BY ADRENOCORCOTROPIN (ACTH) IN RAT ADRENAL GLAND S. Cavallaro, M. Massotto, I. Mocchetti, K. Krueger, S. Casalotti, A. Guidotti and E. Costa, F.G.I.N.; Dept. of Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, DC 20007; I.S. Rome, Italy.

MTR and DBI are highly enriched in steroidogenic cells such as adrenocortical, Leydig, and glial cells. In these cells DBI acts on MTR and stimulates steroidogenesis (Endocrino. 123: 2075, 1991; Papadopoulos V., Neuron, Abst., 1991). We have investigated whether ACTH regulates MTR and DBI expression in rat adrenal gland, tests, cerebellum, and C6-2B glioma cells. Seven days after hypophysectomy the amount of DBI-like immunoreactivity (DBI-li) and DBI mRNA in rat adrenal glands decreased by almost 80%. Similarly binding of the specific MTR ligand, [3H]Ro5-4864, to crude adrenal homogenates, and MTR mRNA in rat adrenal glands increased by almost 80%

588.11

THE ENDOGENOUS POLYPEPTIDE DBI AND OTHER DRUG LIGANDS OF MITOCMDRIAL BENZODIAZEPINE RECEPTOR (MBR) STIMULATE STEROIDogenesis BY C6-2B GLIOMA CELL MITOCHONDRIA. V. Papadopoulos, F. Guarneri, K.E. Krueger, A. Guidotti, and E. Costa, Dept. of Anatomy and Cell Biology and F.G.I.N., Georgetown University School of Medicine, Washington, DC 20007.

Mitochondrial preparations of C6-2B cells convert 22(R)-hydroxysterol to pregnenolone by cholesterol 22-desmolase. Immunoblotting confirmed the presence in C6-2B cell extracts and intact mitochondria of relatively high levels of cytochrome P-450 cholesterol side-chain cleavage enzyme. Furthermore, MTR and diazepam binding inhibitor (DBI), that are operative in mitochondrial steroidogenesis regulation are abundant in C6-2B cells. Pregnenolone synthesis in C6-2B mitochondria is stimulated by nanomolar concentrations of Ro5-4864, PK11195, or DBI as well as by 1-tyrosine and the amino acid mixture (2.66 +/- 1.7 vs. 1.09 +/- 0.62 μM). DBI-stimulated TSH secretion was again more dramatically increased following 1-tyrosine plus amino acid mixture (4.05 ± 7.40 μM) and 1-tyrosine (15 g/Kg, twice daily) reduced the decrease in adrenal DBI-li, DBI mRNA, adrenal binding sites and MTR mRNA caused by hypophysectomy. The regulation of DBI and MTR expression by ACTH is specific for adrenocortical gland and was absent in tests and cerebellum. Administration of 100 M ACTH to C6-2B glioma cells cultured in serum-free medium failed to change the content of DBI-li and MTR mRNA.

588.12

PURIFICATION AND CHARACTERIZATION OF DIAZEPAM BINDING INHIBITOR (DBI) PROCESSING PRODUCTS. E. Slobodyansky, C. Eskalante and B. Martin*. Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007; 54NH, Bethesda, MD 20892.

DBI is a protein with multiple biological actions that is located in selected neurons, glia, steroidogenic cells, and in pancreatic islets. DBI is a potent inhibitor of protein kinase C, depresses food intake and body weight, and has other actions. DBI has been shown to be reduced in several diseases. DBI is cleaved by two proteases, a calcium-dependent carboxypeptidase and a metalloproteinase. The calcium-dependent carboxypeptidase is inhibited by Ro5-4864, PK11195, or DBI. This cleavage releases inactive DBI fragments that cannot inhibit protein kinase C. The metalloproteinase is not inhibited by Ro5-4864, PK11195, or DBI. However, these products cannot inhibit protein kinase C. The metalloproteinase is not inhibited by Ro5-4864, PK11195, or DBI. However, these products cannot inhibit protein kinase C.
OF LATE EMIGRATING AVIAN NEURAL CREST CELLS.

Partial restriction in the developmental potential

Kristin B. Artinger* and Marianne Bronner-Fraser. Developmental Biology Center, University of California, Irvine, CA 92717

last emigrating cells moving dorsolaterally (Serbedzija et al., 1989). In culture, early migrating (“young”) neural crest cells prepared from stage 20-21 embryos, when the last neural crest cells catecholamine-containing cells were found. Because the culture cells were injected into young chick embryos during the early stages of development, “older” neural crest cells. These results suggest that late emigrating neural crest cells have a more restricted developmental potential than early migrating neural crest cells. (supported by USPHS HD-25138)

FUNCTION OF THE DROSOPHILA SINGLE-MINDED GENE AND DEVELOPMENT OF THE CNS MIDLINE

J. R. Nambo, J. O. Lewis*, and S. T. Crews. Dept. of Biology, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

The midline nerve cells of the Drosophila embryo possess unique anatomical, developmental, and functional properties; they play an important role both in the formation and function of the CNS. We are characterizing the development and function of these cells via molecular, genetic, and cellular biological approaches. The single-minded (sim) gene is specifically expressed in the midline cells and we have shown via genetic analyses that sim is required for proper midline cell differentiation and activation of “downstream” midline gene products. It has been determined that sim is a member of the basic-helix-loop-helix family of transcription factors, strongly suggesting that sim acts to directly regulate midline gene transcription. We investigated the effect of ectopic sim expression on midline development and gene expression by generating transgenic flies in which the expression of sim gene is driven by the hsp70 promoter. This heat-shock sim construct allows us to induce ectopic sim expression during different developmental stages. Early induction of sim protein results in defective nervous system formation as well as ectopic expression of midline genes. The data indicate that sim is capable of directing other cell types to exhibit midline-like properties and may function as a master regulator of the midline lineage.


We have performed in vitro clonal analyses to elucidate changes in the temporal and spatial patterns of pluripotent and apparently committed precursor cells in DRG and SG. Previous in vitro clonal analysis of neural crest cell differentiation indicated that all sympathoadrenal cells and some sensory neurons originate from pluripotential neural crest cells (M. Sieber-Blum, Science 243, 1609, 1989). Our present data suggest the following. 1) All four classes of pluripotent and apparently committed cells found in the migrating neural crest are also present in young DRG and SG. 2) The proportion of pluripotent cells decreases with increasing age of the embryo. 3) Both types of DRG cells contain an at least bivalent, possibly “genomic” precursor that persists at least halfway through the incubation period, albeit at a 10-fold lower concentration in the SG than in the corresponding DRG. 4) There is a subset of early-differentiating sensory neurons in the DRG, explaining and confirming previous studies by us and others. These data support the notion that the life-span of pluripotent cells within the neural crest as well as in DRG and SG correlates well with the timing of proven and putative location-specific environmental cues influencing the ultimate phenotype generated by these pluripotent cells. Supported by USPHS grant HD21423 and a Research Grant from the Familial Dysautonomia Foundation (M. S-B) and NIH Training Grant GM07892-09 (R.S.D.).

Cell to cell interactions may participate in determining the fate of cells in the central nervous system. We have used whole-cell patch clamp recordings to characterize the input properties of cortical neurons originating from different clonal populations. In this experiment, we tested the hypothesis that some aspects of the adult mammalian cortex might already be predetermined within the cell lineage of postmitotic cells. To determine the mechanisms that control the determination of cell phenotype in the developing central nervous system, we are using dissociated cell cultures of Med, New Haven, CT. In this experiment, we are determining the phenotype of the isolated dissociated cells using both immunocytochemical and neurochemical methods.


We hypothesized that such early predetermination might manifest itself in heterogeneous proliferation kinetics among individual cell lineages within the developing retina. In the developing mammalian telencephalon there is evidence to suggest that some aspects of the fate of forebrain cells are predetermined while they are still proliferating in the ventricular zone (Krubel et al. 89, Soc. Neurosci. Abstr. 241; Fehlhaber et al. 90, Devel. Biol. 110:70; Barde&Levitt, 91, J. Neurosci. 11, 519-533).

In the developing telencephalon, the major cytoarchitectonic subdivisions of the neocortex and the cerebellum may occur early in development before neurons attain their definitive positions (Lo Turco et al. 91, Science). Here we report the early segregation of cell lineages within the developing telencephalon. GABA antisera were used to immunolabel frontal and occipital lobes at all ages examined, including E41 when the cortical plate is as yet only 2-3 cell layers wide. GABA-containing neurons were also present in the ventricular and subplate zones, and some of these cells had present in the cortical plate in both frontal and occipital lobes at all ages examined, including E41 when the cortical plate is as yet only 2-3 cell layers wide. GABA-containing neurons were also present in the ventricular and subplate zones, and some of these cells had
We wished to relate digitized cortical and hippocampal EEG. Images were averaged over 1 min to determine the significance of the comparisons using an alpha of .05. Cocaine eliminated background activity. A multiplication also was performed to amplify varying, depending on the preadministration state of the animal; typically hippocampus; this decline began as early as 1 min, with the maximal decline baseline levels requiring approximately 15 minutes.

Concentrated \(^{[25]}\)WGA injected percutaneously into rat forelimb or hindlimb muscle in various microfiber quantities using methacrylate to seal the puncture site was allowed three to ten days to distribute after which animals were sacrificed, perfused, and 25 different tissues including blood, lymph nodes, liver, lung, muscle, ipsilateral and contralateral peripheral nerve, spinal root/dorsal ganglia, and various spinal cord segments were collected, weighed, and assayed in a gamma counter.

Excluding the injection site (as could be done in a clinical image) WGA concentrations (as counts per min/mg) in ipsilateral and ganglia were up to five to ten times greater than that in any other tissue in the same animal when injection technique and dose were optimized. Spinal cord segment concentrations were greater than even in non-neural tissue except liver. These results clearly show that biodistributions of axonal tracers can be achieved which may permit clinical imaging of phenomena involving axonal transport in humans if PET or PET detectable labels can be delivered in adequate quantities. Direct application of axon transport imaging in the evaluation of nerve compression, spinal cord injury, and neuropathy as well as other reanimations of a variety of transport related basic neuroscience techniques into the clinical realm are possible.

**DYNAMIC INTRACELLULAR CALCIUM COMPARTMENTS: THREE DIMENSIONAL CONFOCAL MICROSCOPY OF FLO-3 IN PC12 CELLS.** T. M. Miller and D. S. Puro, Division of Developmental and Molecular Neuroscience, Laboratory of Playfair Neuroscience Unit, The Toronto Hospital, 399 Bathurst St. Toronto, ON Canada MST 2S8

A confocal microscope (Biorad MRC-600) and an ICAR 80.8 Volume Investigation workstation were used to study the three dimensional and optical properties of the calcium indicator dye fl-o in NGE activated PC12 cells. In addition to its expected cytoplasmic distribution fl-o was also found to label the nucleus and a variety of subcellular organelles. Labelling with rhodamine 123 identified one class of organelles labelled by fl-o as mitochondria. Other organelles are as yet unidentified. In cells loaded with fl-o in the presence of serum at 37 degrees, rest calcium levels were significantly higher in the mitochondria than in the cytoplasm: on average calcium levels in the cytoplasm were 74±8 nM (n=21 cells), compared to 129±11 nM (n=20 cells) in the mitochondria. Depending on the cytoplasmic or extracellular calcium levels the calcium ionophore A23187 or removal of extracellular calcium indicate that fl-o can act as a calcium indicator in all compartments examined. Furthermore, calcium dynamics appears to be different in different subcellular compartments.


To explore the possibility of PET or MR imaging of axonal transport in humans, a series of studies were undertaken in order to learn whether tracer concentrations in nerve and among other tissues could achieve imageable relative distributions. The underlying principle is an extension of the theory of transport from small intramembrane molecules. Nuclear injections of wheat germ agglutinin (WGA) are used to selectively label and distinguish spiral cord neurones innervating long-closely juxtaposed muscles.

Concentrated \(^{[25]}\)WGA injected percutaneously into rat forelimb or hindlimb muscle in various microfiber quantities using methacrylate to seal the puncture site was allowed three to ten days to distribute after which animals were sacrificed, perfused, and 25 different tissues including blood, lymph nodes, liver, lung, muscle, ipsilateral and contralateral peripheral nerve, spinal root/dorsal ganglia, and various spinal cord segments were collected, weighed, and assayed in a gamma counter.

Excluding the injection site (as could be done in a clinical image) WGA concentrations (as counts per min/mg) in ipsilateral and contralateral peripheral nerve, spinal root/dorsal ganglia, and various spinal cord segments were collected, weighed, and assayed in a gamma counter.

**IN-VIVO IMAGING OF INTRACELLULAR ION CONCENTRATIONS IN RAT BRAIN NEUROCITOS IN DYNAMIC STUDIES.** U. Dittman, A. Villinger, A. Tem, G. Srin, K.M. Ehm, Dept. of Neurology, University of Munich, Munich 8000 Munich 70, F.R.G.

We tested the feasibility of in-vivo loading and imaging of brain cells in situ with ion-sensitive dyes. Anesthetized and ventilated Wistar rats were equipped with a closed cranial window (dura mater removed), and the brain surface was superfused with the pH-sensitive dye BCECF-AM (450 nM) and/or the Ca\(^{2+}\)-sensitive dye Calcium-Crimson-AM (150 nM). Both dyes were observed through a Fluorescence Confocal Laser Scanning Microscope (CLSM, Biorad MRC-600) with an Ar/Kr-laser (488/560 647 nm) as a light source. Image formation was triggered to respiratory phase. Leakage of dyes was assessed by the loss of intracellular signal over time and with spectrally-fluorescent determination of dye concentrations in the outwash CSF. At the end of the experiment, calibration of intracellular pH (pH_i) was performed by collecting concentration calibration buffers titrated to pH 6 and 8 respectively (nigericin/high potassium method). Autofluorescence of the brain was low. On optical sections dye related signal was retrieved up to 200 μm beneath the cortical surface, single dye loaded cells could be visualized down to 50 μm. Double labelling allowed simultaneous imaging of pH_i and Ca\(^{2+}\) related signal. The rate of photobleaching was low, and leakage of the dye was less than 15 % per hour. Motion artifacts and changes in brain geometry with experimental manipulations (i.e. clamping) impeded the accuracy of the measurements. In these experiments we have shown that 1) loading with AM-dye-esters, 2) CLSM imaging, and 3) pH_i/pCa-related imaging of brain cells in situ is feasible.


Cocaine induces marked changes in neuronal discharge of limbic structures, including prefrontal cortex or nucleus accumbens, an effect maintained for periods of 60-150 min. We wished to relate electrophysiological activity with local metabolic actions of cocaine, and thus examined the course of local metabolism in the dorsal hippocampus following intravenous cocaine delivery using a cohercon-image technique. Electrocorticography recorded EEG, eye movements, and diaphragmatic activity in three cats under surgical anesthesia, and a 1.6 mm diameter image probe was placed over the dorsal hippocampus. After recovery, a 2.5 mg/kg intravenous cocaine was administered following an iv saline control injection, and the time course of successive frames of reflected 700 nm optical activity was examined for 30 minutes. Frames were collected together with direct optical and hippocampal EEG. Images were averaged over 1 min epochs, and a pre-cocaine epoch was subtracted from each subsequent epoch to eliminate baseline and flow and local metabolism. A multiplicity of image differences, and ANOVA procedures for each individual pixel were used to determine the significance of the comparisons using an alpha of .05. Cocaine induced a rapid diminution in optical activity from the dorsal hippocampus; this decline began as early as 1 min, with the maximal decline varying, depending on the prednimination state of the animal; typically, maximal declines occurred at 2 min post administration, with recovery to baseline levels requiring approximately 15 minutes. Supported by NIDA DE07122. G.P. is supported by a Howard Hughes Medical Institute Predoctoral Fellowship.
A substantial fraction of retinal ganglion cells survive to a higher extent is caused by 'aberrant' myelination, or degeneration of retinal ganglion cells (GC). Our study examined whether myelinated axons survive to a higher extent, is ordered on the basis of their anterograde to the cut. The surviving GC bodies and motoneurons regenerating correctly down the motor branch were much more numerous and present in most of the brainstem areas covered with myelin. Thus, after degeneration of about 90% of the axons, oligodendrocyte myelin 'aberrant' cellular elements were covered with myelin. Then, after degeneration of about 90% of the axons, oligodendrocyte myelin 'aberrant' cellular elements were covered with myelin. The proportion of the retina was inferred from the lesioned retina of the intact retina. Whether the enhanced proportion of myelinated axons is caused by 'aberrant' myelination, or whether myelinated axons survive to a higher extent, is still open. It remains to be shown why some GC are capable of surviving and whether they could regenerate their axon in a 'suitable' environment.

The video microscopic images of the serial sections (around 200) were sequentially acquired to a computer (Hamamatsu Photonics C2000). The acquired images were processed as 16-bit gradient natural images without any human handlings such as tracings or binary slicing. These images were reconstructed by our computer programs to produce hypothetical pictures of the stained neuron viewed from any angles other than actually observed. By presenting these calculated pictures of different view angles sequentially on the video screen, full rotation of the three-dimensional natural-looking image of the neuron was produced satisfactorily. We also precisely determined the volumes, surface areas and lengths of the cell body, axons and neurites of the stained neurons three-dimensionally.

The video microscopic images were used to produce hypothetical pictures of the stained neuron viewed from any angles other than actually observed. These images were reconstructed by our computer programs to produce a virtual three-dimensional image of the neuron. This high-pass result is then automatically thresholded to separate the grains from the background.

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RAT PERIPHERAL NERVE REGENERATION ACROSS 18-mm GAPS AND
Bailey1, M.E. Eichler2, A. Villadiego*, K.M. Rich2,3, Departments of growth factor (NGF, 1 mg/ml), were sutured between the severed ends of sciatic HRP-labeling demonstrated that significantly more sensory and motor neurons incidence of cables across the 18-mm gaps. The number of myelinated axons formation -- the initial step for regeneration, all the groups had at least a 60% FB, LN, FB/LN, NGF, or cyto-C were added to silicone chambers forming 5-μm. augmentation of Schwann cell migration. As above, sterile saline solutions of FB, LN, FB/LN nerve grafts on the survival and axonal growth of retinal ganglion cells (RGCs) in adult rats. Normal sciatic nerves (group A) and sciatic nerves which had been crushed one week before transplantation (group B, 'conditioned' grafts) were used as grafts. The nerves were removed and cultured to the proximal stump of intraorbitally transplanted optic nerves. Neuronal survival and axonal growth were determined by counting the numbers of surviving DiI prelabeled RGCs, cresylviolet stained RGCs and the numbers of axons which had grown into the grafts three and six months after transplantation. Counting of axons was performed by combined use of light- and electronmicroscopy.

We observed that the use of conditioned grafts (group B) significantly enhanced RGC survival and axonal regrowth as compared to normal grafts at 3 months after transplantation. Six months after grafting, RGC survival (as determined in DiI stained retinas) and axonal growth were not significantly different in both groups. When cresylviolet stained retinas were analysed, significantly more RGCs were survived in group B compared to animals with normal grafts. These results suggest that the functional status of a peripheral nerve used for grafting in the CNS influences neuronal viability and axonal regeneration especially during the first 3 months after grafting. Long-term RGC survival, however, may be determined by functional reconnection of regenerating RGC axons rather than by the graft itself. G.W.E. is a fellow of the Boehringer Ingelheim Fonds.

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Sound-localization deficits induced by lesions in the owl's auditory space map. A. C. D inarello2* 1Brown University, Providence, RI and 2Tufts University and New England Medical Center Hospital, Boston, MA.

All of the African mormyrid fishes have specialized ears containing a gas-filled bladder within each labyrinth, and the direct coupling of an otolithic organ (saccule) to this bladder. Specimens were collected in the wild, and their auditory nuclei were studied using a Biorad Confocal Imaging System. A


decayed IL-1ra (dIL-1ra) combined with bovine serum albumin (BSA) was crushed unilaterally in anesthetized rats for 90 s. The wound was irradiated transcutaneously, daily. Rats were fixed by intracardiac perfusion on days 5 and 11 and sections were processed for immunofluorescence. Fluorescence levels of CHAT were quantified using a Blorad Confocal Imaging System. A significant increase (p < 0.05) in CHAT fluorescence intensity/μm2 was detected in motoneurons of irradiated versus nonirradiated injured rats at 5 and 11 days. Whether this increase in CHAT levels late with decrease in the initial degenerative events or signifies an earlier return of acetylcholine synthesis is being determined.

591.12

LOW ENERGY LASER IRRADIATION ALTERS IMMUNOFLUORESCENCE OF CHOLINE ACETYLTRANSFERASE (CHAT) IN REGENERATING RAT FACIAL NEURONS. J.J. ANDERSON, J. BURKE, and L. W. BORKE, Dep. of Anatomy and Cell Biology, UCONN, Bethesda, MD 20814.

The authors have shown that low energy He-Ne laser irradiation (632nm, 8.5mW, 90 min) increases the rate of facial nerve regeneration after crush injury. CHAT decreases during the first week post-crush and returns to normal levels by 20 days in motoneurons (Burke and Borke, Anat. Rec., 226:24A, 1990). The purpose of this study was to determine if irradiation could effect CHAT changes during denervation and regeneration. The facial nerve was crushed unilaterally in anesthetized rats for 90 s. The wound was irradiated transcutaneously, daily. Rats were fixed by intracardiac perfusion on days 5 and 11 and sections were processed for immunofluorescence. Fluorescence levels of CHAT were quantified using a Blorad Confocal Imaging System. A significant increase (p < 0.05) in CHAT fluorescence intensity/μm2 was detected in motoneurons of irradiated versus nonirradiated injured rats at 5 and 11 days. Whether this increase in CHAT levels late with decrease in the initial degenerative events or signifies an earlier return of acetylcholine synthesis is being determined.

591.11

INTERLEUKIN-1 RECEPTOR ANTAGONIST RELEASED FROM A POLYMERIC GUIDANCE CHANNEL IMPAIRS PERIPHERAL NERVE REGENERATION. P. Aeberscher1, V. Guénard1, C.A. Dinarello2* 1Brown University, Providence, RI and 2Tufts University and New England Medical Center Hospital, Boston, MA.

Schwann cell-mediated nerve growth factor (NGF) effects play a key role in regenerative processes in the PNS. Upon injury, interleukin-1 (IL-1), a cytokine produced by macrophages and Schwann cells, activates macrophages also receptor antagonist (IL-1ra), a true antagonist which binds to IL-1 receptors without inducing any IL-1 effects. Ethylene vinyl acetate (EVA) copolymer tubes releasing controlled amounts of either IL-1ra or deactivated IL-1ra (DIL-1ra) combined with bovine serum albumin (BSA) were implanted subcutaneously into the skin around a 4 mm nerve gap. Channels releasing BSA only were used as controls. Four weeks postimplantation, the nerves grew along the tubes. The deficits in microfascicles containing unmyelinated and myelinated axons with their associated Schwann cells. However, tubes extending through BSA-IL-1ra-releasing channels contained significantly less unmyelinated and myelinated axons compared to controls receiving BSA- or BSA-DIL-1ra-releasing channels. This study demonstrates that a true antagonist to IL-1 receptors impedes peripheral nerve regeneration and suggests that drug-delivery devices may aid nerve regeneration by releasing stimulatory and/or inhibitory molecules.

591.2

"GLYCINE RECEPTOR" IMMUNOSTAINING SITES MARK CLASSES OF AUDITORY VESTIBULAR NEURONS. J.C. ADAMS ENT Dept., Mass. Eye & Ear Infirmary, Boston, MA 02114.

A monoclonal antibody (KM) raised against strychnine binding sites recognizes sites that do not bind strychnine nor contain much glycine. One example is an inclusion body within the cytoplasm of auditory and vestibular neurons. These cells are in the MNTB, primary vestibular neurons, octopus cells, spherical cells in the VNLL, and spherical cells located caudal and ventral to the LSO. Three of these cell classes are immunoreactive for glycine. Octopus cells and Scarpa's ganglion cells are not. One trait shared by cells containing inclusion bodies is the sparsity of immunoreactive sites for the "glycine receptor" on their surfaces. This suggests that the inclusion bodies are probably not sites where the receptor is made. The role of the inclusion bodies is obscure, but their presence can be used for identifying cell classes. For example, with the inclusion body as the defining trait of octopus cells: humans have very many; rats have many; rodents have few. This is another example of a closer similarity of cat and human auditory systems as contrasted with rodents. Supported by NIH grant DC00269-07.

592.3

Sound-localization deficits induced by lesions in the owl's auditory space map. H. Wagner Max-Planck-Instit für biologische Kybernetik, Spemannstrasse 34, D-7404 Tübingen, FRG

Barn owls possess a map of auditory space. The map is synthesized in the external nucleus of the inferior colliculus (ICx) and then projected to the optic tectum (OT). Azimuths of owls were recorded before and after lesioning ICx and/or OT. After focal electrolytic lesions, 1 observed increased response latencies, reduced turning amplitudes and an increased latency to turn away from the source. These deficits occurred at azimuths expected from the physiological data obtained at the lesion site before passing current. The deficits extended over weeks and were obvious from 3 to 20 days after the lesion. After this transient phase, mean response latency remained increased in 1 of 6 cases, and the number of turns with very short latency decreases. Thus, this is the first unambiguous demonstration, for sound localization, of a deficit covering a locally well-circumscribed area within a hemisphere. Since the major lesion in this case was a small one, the occurrence of a deficit in the external nucleus of the inferior colliculus (ICx) and then projected to the optic tectum (OT) is not unexpected. Two other cases of similar deficit latencies are excluded that the animals learned to respond tactically. The deficits were also not due to motor impairment, because immediately after the lesion, the animals responded normally to stimulation from outside the well-circumscribed region of the lesion.

592.2


The auditory event-related response detected magnetically about 100 msec following the onset of a tone burst with a long interstimulus interval (ISI) reveals a component of neuronal activity that overlaps in time with the response in primary auditory cortex (N100m). We call this activity the "latent" component (L100m) since it requires a much longer ISI than N100m to reach a full response amplitude when stimuli are presented at an interstimulus interval of 0.8 to 16 sec. The resulting ISI dependence for L100m is exponential with an exponential time constant of 1.5 sec longer than for N100m, the latter ranging from 1.5 to 3.5 sec across individuals. The locations of the neuronal activity that produce L100m are deduced from the field pattern by a least squares fit of 2 current dipoles. By comparison with MRI, both were found to lie on the supratemporal plane with the source of N100m lying in primary auditory cortex and the source of L100m lying about 1.5 cm more posterior and 1 cm more inferior than that of N100m. The second location was identified as the auditory association cortex.

**Supported in part by AFOSR grant AFOSR 90-0221.
592.5 DISRUPTION OF INTERHEMISPHERIC INTEGRATION OF COMPLEX AUDITORY INFORMATION FOLLOWING LOCAL HEMICHEMIAION INTO THE POSTERIOR BODY OF THE CORPUS CALLOSUM. M.L. Trans, F.E. Murdock, and M.S. Ozelius.

Program in Cognitive Neuroscience and Division of Audiology, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756

In rodent hemichemias, the posterior body of the corpus callosum contains interhemispheric projections of auditory cortical neurons in the supratemporal plane and posterior superior temporal cortex (Panda and Seltzer 1986). To our knowledge, no one has indicated the difficulty of the callosum by means of single neurons. In this study, the excitatory bandwidth topography of auditory projections from a single neuron in the supratemporal plane was recorded in response to narrow-band stimuli in humans. The results indicate that the neurons from the supratemporal plane and the posterior superior temporal cortex were distributed in a topographic manner in the callosum. Similar findings were obtained for the more anterior auditory cortex of the supratemporal plane. These findings indicate that: 1) the posterior body of the corpus callosum mediates the interhemispheric integration of physiological information; and 2) the topography of callosum auditory projections is homologous in human and nonhuman primates.

Supported by NIH POI 17778 & ROI NS25286


In this study of the adult and early postnatal ferret lateral superior olivary nucleus (LSO), it was observed that cells giving rise to the ipsilateral and contralateral ascending projections were segregated spatially within areas of similar frequency representation. These findings suggest that the homotopic segregation was more striking in the ferret LSO than the heterotopic variation that has been previously reported. The cellular layer of the intermediate limb of the LSO was divided arbitrarily into eight parallel lanes from core to margin. Labeled projections on the ipsilateral and contralateral side with respect to tracer injections in the inferior colliculus were mapped and position planes in a horizontal plane of the LSO were occupied. There was a clear tendency for ipsilateral projection cells to occupy marginal lanes and contralateral projection cells to occupy core lanes. Approximately two-thirds of the ipsilateral projection cells were found in the two inner and outer lanes compared to only one-third of the contralateral projection cells. The distribution curves varied from case to case and limb to limb, probably in part due to the irregular orientation of the limbs of the LSO with respect to standard anatomical planes. Some homotopic segregation in the LSO was evident at birth indicating that this rule of organization may be in place at least four weeks prior to the onset of hearing. Cells marked by other phenotypic traits revealed similar homotopic segregation. Glycine- and GABA-immunoreactive cells were most frequent in the marginal lanes. Similar immunoreactive cells were used in the developing LSO to homotopic gradient of immunoreactive puncta in the LSO was apparent suggesting that the output but not necessarily the input to the LSO may be segregated. Further studies are underway to address these hypotheses.

Supported in part by NIH grant DC00335.

592.7 ANIMAL BEHAVIORAL MODEL OF HALLUCINATIONS IN RATS. P.J. Pitcher and J.F. Brennan. Department of Surgery, Univ. Maryland Sch. Medicine, Baltimore, MD 21201; and Dept. Psychology, Univ. of Massachusetts/Boston, MA 02125.

An animal model of phantom auditory perception has been tested on saline-pretreated rats. The auditory system (Cohen, Behavioral Neurosci. 102:811-822, 1988) and involves continuous exposure of rats to background noise or light with offset of the background serving as a conditioned stimulus (CS). The model has been expanded to accommodate visual and auditory hallucinations by introducing complex background of pulses of tone or light of random length presented randomly, with the offset of the background serving as CS. Pulses of light/tone of different traits superimposed on background. Findings in behavior consistent with our previous findings.

The additional use of scopolamine (0.5-0.75 and 1.0 mg/kg/day) or scopolamine-induced visual hallucinations, produced changes in extinction among groups trained with visual modality, but not with the auditory modality, in a dose-dependent manner. These results are consistent with the postulate that scopolamine induces visual hallucinations in rats, detected by our paradigm. This offers a possibility for creating a behavioral animal model for schizophrenia. (NIH NIMH Grant DC00449)

592.8 IDLING 10-Hz RHYTHM IN THE HUMAN AUDITORY CORTEX? R. Hari, Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland.

The 10-Hz alpha rhythm in the visual cortex and the corresponding mu rhythm in the somatomotor cortex are identified as low-frequency rhythms of the sensory systems; they are blocked by visual and tactile stimuli, respectively. We have recorded magnetic spontaneous activity with a 24-channel superconducting quantum interference device (SQUID) gradiometer to find out whether the human auditory cortex is idling an 10-Hz rhythm. Magnetic 10-16 Hz rhythm was observed over the temporal regions. It was dampened by auditory stimuli but it did not decrease when the eyes were opened or when the head was turned. The field patterns over the auditory cortex could be satisfactorily explained by equivalent current dipoles, situated 2 cm from the source of the 100-msec auditory evoked response, generated in the supratemporal auditory cortex. During light drowsiness, when the occipital electric alpha became discontinuous and spread to more anterior scalp locations, the temporal magnetic 10-Hz rhythm accentuated and resembled the frontocentral electric activity. Since currents at the superior surface of the temporal lobe would produce the maximum potentials in the frontocentral midline, the 'spread' of electric 'alpha' during drowsiness might reflect activity of the temporal-lobe 10-Hz generators. The results suggest that each sensory projection cortex has its own idling rhythm.


592.9 TOPOGRAPHY OF INTENSITY ENCODING BY SINGLE NEURONS IN CAT PRIMARY AUDITORY CORTEX. M.L. Sutter and C.E. Schreiner. Coleman Laboratory, Dept. of Otolaryngology, University of California, San Francisco, CA 94143-0732.

The topography of the sharpness of amplitude tuning (monotonicity) of single neurons was studied within the inferior frequency domain of high frequency (CFs > 4 kHz) primary auditory cortex (AI). To pool data across animals, the multiple unit monotonicity (Sutter et al., 1988) and excitatory bandwidth with bandwidth maps were used as topographical frames of reference (Sutter and Schreiner 1991). CFs were measured at the level of the monosynaptic excitation and inhibition. The monotonicity map consisted of two nonmonotonic (sharply tuned for amplitude) areas along the inferior frequency (roughly dorsal-ventral) domain of AI. Monosynaptic response profiles were obtained in units (50%) and multiple (30%) recordings. In ventral AI, the spatial distribution of single neuron monotonicity ratios (response at highest intensity divided by response at lowest intensity) paralleled the shape of the multiple unit spatial distribution. In dorsal AI, pooled single neurons displayed no clear topographic order with respect to amplitude tuning. The two findings suggest that the scatter of intensity thresholds for single neurons was observed, implying that nonmonotonic multiple unit areas are a result of tightly overlapping intensity receptive fields for single units. These data indicate that projecting AI dorsal (AI(d)) and ventral (AI(v)) regions, as supported by the excitatory bandwidth topography, is justified; however, projection to the inferior nonmonotonic area lying at the AI(d)/AI(v) border need to be considered in more detail.

The ventral multiple unit nonmonotonic area roughly aligned with the area of sharply frequency tuned units described by Schreiner et al. (1991). Neurphysiol. 64: 1142-1160. This region, with low intensity thresholds, narrow intensity tuning, and narrow frequency tuning corresponded to an intensity floor for low intensity narrow-band stimuli, just above the background noise. (This work was supported by NINDS/NIH grant NS04141, NIH training grant GM08115, the Coleman Fund, and Hearing Research Inc.)
592.1


In a previous study (Robertson & Irvine, J. Comp. Neurol. 282:456-471, 1989), restricted cochlear lesions in adult guinea pigs were found to result in a reorganization of the representation of the lesioned cochlea in the contralateral auditory cortex. One question raised by this result concerns the effect of this reorganization on the representation of the normal (ipsilateral) cochlea in that cortex. This question was examined in adult cats in which restricted unilateral cochlear lesions were performed by direct mechanical trauma. Following a 2-6 month post-lesion recovery period, the animals were reasseditized, the extent of the peripheral hearing loss in the lesioned ear was established by means of the compound action potential audiogram, and the frequency organization of primary auditory cortex (AI) contralateral to the lesioned ear was determined using conventional microelectrode mapping techniques. In the representation of the lesioned cochlear cochlea the region deprived of its normal input by the lesion was partially occupied by an expanded representation of the frequency at the edge of the lesion, but the ipsilateral frequency map in this region showed a normal frequency progression. Evidence on the thresholds at their new characteristic frequencies of neuronal responses in the enlarged contralateral representation indicated that the expanded representation reflected a reorganization of cortex rather than simply residual drive at frequencies unaffected by the lesion. The lack of change in the ipsilateral frequency map results in a loss of the frequency variance between the two frequency maps, and has implications for the mechanisms underlying auditory cortical reorganization.

PRESYNAPTIC MECHANISMS: ION CHANNELS

593.1


Aplysia sensory neurons contain "N-like" Ca++ channels which participate in transmitter release, and dichydroprimidine-sensitive "L-like" Ca++ channels which do not contribute to release (Edmonds et al., 1990). 5-HT produces presynaptic facilitation of the Aplysia sensory-motor neuron synapse, and increases the sensory neuron [Ca++], transient during action potentials. The increase in the [Ca++] transient is due to two processes: 1) indirect modulation whereby [Ca++] transient closure and resulting spike broadening increase influx through both N and L channels, and 2) a direct increase in Ca++ current. Since only N Ca++ current contributes to presynaptic facilitation (Edmonds et al., 1990), we have used two-[Ca++], measurements with or without nitrendipine to assess the relative contributions of N and L current to the [Ca++] transient and its modulation by 5-HT. N Ca++ current increased 11 ± 3% in the absence of nitrendipine, and increased 17 ± 4% in the presence of nitrendipine (p < 0.01; two-tailed t-test) in the amplitude of [Ca++], transients in the cell body produced by action potentials. However, in the presence of 10 μM nitrendipine (which blocks the 5-HT-induced increase in current by 87 ± 4%), 5-HT caused only a 28 ± 8% increase in [Ca++] transient amplitude (n = 9), significantly different both from control (p < 0.01) and from 5-HT alone (p < 0.01). The 28% increase presumably reflects increased Ca++ influx via the N channels due to spike broadening. In nitrendipine, the amount of spike broadening produced by 5-HT was roughly correlated with the increase in [Ca++] transient amplitude. As shown in the accompanying abstract (Elot et al., 1991), a similar increase in Ca++ influx is seen in presynaptic sensory neuron processes in 5-HT + nitrendipine, supporting its role in presynaptic facilitation.

593.2


The accompanying abstract (Blumenfeld et al., 1991), demonstrated that 5-HT increases Ca transients in Aplysia sensory neurons both by direct modulation of the L-type Ca current and by action potential broadening, which indirectly enhances influx through both L- and N-type Ca currents. Here, we examine the contributions of these two mechanisms to the increase in Ca influx in synaptic terminal regions. Serotonin produced a 39 ± 9% (N = 5) increase in the [Ca++] transient in regions of the sensory neuron making contact with a postsynaptic motor neuron, and facilitated the EPSP by 47 ± 16%. Nitrendipine reduced this increase by 65%, but did not reduce facilitation of the EPSP. In separate cultures, nitrendipine by itself reduced the Ca transient to 80 ± 5% of control (N = 3). The latter effect suggested that depression might be mediated by cyclic nucleotides. Serotonin produced a 73 ± 7% reduction of the Ca transient in nitrendipine, and its modulation by 5-HT failed. These results indicate that direct modulation of the L-type current contributes roughly 2/3 of the enhancement of Ca influx by 5-HT, but this does not contribute to facilitation. The remaining enhancement of Ca influx occurs through N-type Ca channels as an indirect consequence of spike broadening. There was a significant correlation between enhancement of Ca influx via the N-type channels and presynaptic facilitation. These experiments also provide some evidence that the two classes of Ca channels are differentially localized.

593.3

SYNAPTIC PLASTICITY OF A DUAL-ACTION POTENTIAL MODIFIED BY METABOLITES OF ARACHIDONIC ACID IN APLYSIA. E. Shapiro. Howard Hughes Medical Institute, New York, New York 10032.

In the abdominal ganglion of Aplysia, an identified cluster of interneurons called L32 cells makes a dual-action synaptic potential onto L14 cells, motor neurons controlling the defensive release of ink. The dual-action consists of a fast component mediated by a polypeptide neurotransmitter, and a slow component mediated by a neurotransmitter which is a metabolite of arachidonic acid (AA). This slow neurotransmitter action is blocked by specific antagonists of AA metabolism which produce the active 12-lipoxygenase products 12-hydroxyprostaglandin E2 (PGH2), 12-HETE and 12-hydroxy-5,13-epoxyeicosatrienoic acid (5,13-EPETE) and 12-hydroxy-eicosatetraenoic acid (12-HPETE) and 8-hydroxy-9,11-epoxyeicosatrienoic acid (8,9-ETE). It is not known whether AA metabolism produces only the 12-lipoxygenase products or if 5,13-ETE and 8,9-ETE are also produced (K. L. M. Sankey, personal communication). We have obtained evidence that this depression may be mediated by the neuromodulator adenosine. Synaptic depression was assessed by measuring EPSP amplitudes in 8 neurons evoked by stimulating A neurons at 3 min intervals and by changes in potentiated EPSP amplitudes (Slow Developing Potentiation, SDP). A-SDP, Fredman, 1988) produced by 2 sec 20 Hz trains in the A neurons. The L32-L14 dual-action synapse displays quite variable strength. Within the same L32 cluster different individual cells may produce almost pure excitation of a postsynaptic target cell, or a pure inhibitory postsynaptic potential on the same L14 follower cell. Similar to other dual-action responses, the L32-L14 dual-action is frequency-dependent. At low frequencies of stimulation ( ≤ 1 Hertz) the early excitatory phase predominates, while at higher frequencies of stimulation, the depolarizing phase undergoes synaptic depression, and the slow inhibitory phase predominates. When L32 cells are transected (e.g. 20 Hz stimulation for 20 sec) the slow IPSP component of the synaptic response elicited by a short train of spikes (20 Hz for 1 sec) exhibits post-episodic potentiation. In each ganglion up to seven L32 cells converge on L14. I am currently testing whether activity in one L32 cell may affect the synaptic response of L14 in other L32 cells.

593.4

ADENOSINE MEDIATES SYNAPTIC DEPRESSION IN APLYSIA. S.M. Fredman. Department of Physiology, Meharry Medical College, Nashville, TN 37208.

Low frequency stimulation of the A-B neuron synapse in the cerebral ganglion of Aplysia produces a long-lasting depression. Only 5 spikes presented at 3 min intervals result in a 49±14% (N=10) reduction in EPSP amplitude. I have obtained evidence that this depression may be mediated by the neuromodulator adenosine. Synaptic depression was assessed by measuring EPSP amplitudes in 8 neurons evoked by stimulating A neurons at 3 min intervals and by changes in potentiated EPSP amplitudes (Slow Developing Potentiation, SDP). A-SDP, Fredman, 1988) produced by 2 sec 20 Hz trains in the A neurons. A-SDP, Fredman, 1988) produced by 2 sec 20 Hz trains in the A neurons. Adenosine agonists increased depression. Bath application of 100 μM adenosine (ADO) or 150 μM of the A, agonist, N- cyclohexyladenosine reduced SDP and increased synaptic depression. ADO antagonists decreaased depression. Application of 100 μM D-PSX reduced depression and increased SDP. ADO analogs with phosphodiesterase activity had two effects. At low concentrations, both 100-400 μM IBAX and 200 μM theophylline reduced or blocked synaptic depression. At high concentrations (1-2 mM) they increased it. The latter effect suggested that depression might be mediated by cyclic AMP. Both bath application of DB-cAMP and direct injection of cAMP into A neurons reduced SDP. Adenosine deaminase (1 mg/ml) which hydrolyses ADO significantly reduced depression, indicating that endogenous ADO may contribute to depression of the synapse. These results suggest that the A-B neuron synapse in Aplysia, depression may be mediated by presynaptic inosine autoreceptors acting via a cAMP dependent mechanism. Thus, as in the mammalian CNS, adenosine may also modulate synapses in Aplysia. Supported by grant NS28199 to SMF and RCMI, MBRS and NSF/MRCE grants to Meharry Medical College.
amplitudes were distributed around two values, either around zero or around a given mean amplitude, with a considerable variability (EPSCs, continuous line) in fig.B). The mean amplitude varied considerably between synapses.

A METHOD FOR RELIABLE ACTIVATION OF A SINGLE EXCITATORY SYNAPTIC CA1 PYRAMIDAL CELLS

M. Raastad, Inst. of Neurophysiology, Pb 1104, N-0317 Oslo, Norway.

A reliable single synaptic input is a useful tool for a detailed study of synaptic mechanisms. By stimulating with a small (2-4μm) glass pipette filled with extracellular solution, positioned 100-200um off-centre in the dendritic field of the CA1 cell, small EPSCs of highly variable amplitude (typically < 10 pA) intermixed with no failures. This suggests that: (1) stimuli that release or do not release Ca2+ as it diffused the short distance between the clustered Ca2+- and Na+ channels, (2) there were still some failures, but when records with failures were omitted, the mean amplitude of non-zero EPSCs did not change. When medium pipette contained blockers, including (IC50’s in parentheses) ω-aconitine (5-10μM), 4-aminopyridine (10μM) and tetrodotoxin (0.1μM), nearly tripled the mean amplitude and the variability between the size of the excitatory postsynaptic current (EPSC) and stimulus strength above threshold (T in fig.A). The mean amplitude varied considerably between synapses.

A STUDY OF SYNAPTIC FACILITATION IN CA1 PYRAMIDAL CELLS USING WHOLE CELL REERATURE FROM PALM TREES AND A FEW PRESYNAPTIC FIBERS

J.M. Storm & M. Raastad, Inst. of Neurophysiology, Pb 1104, N-0317 Oslo, Norway.

Pared-pulse facilitation (PPF) is a widespread form of short-term synaptic plasticity, which may be due to residual Ca in the presynaptic terminal following the first impulse. We have used DCFAM, a fluorescent dye, to study the entry and action of Ca2+ in the frog saccular hair cells. We propose that the entry and action of Ca2+ in frog saccular hair cells is a Ca2+-dependent neurotransmitter release in CNS tissues. (supported by NS-28568).

which sodium/proton antiporter or the chloride/bicarbonate exchangers transporters at the platelet surface or alterations in ion gradients. The 5-HT transporter in platelets. Most of the effect of PKC activation N=16, p <.0005). 4-Alpha-TPA was without effect on either the Vmax or K m uptake. A smaller effect on K m was also observed (mean 15% decrease, 2,3-dione (CNQX, 100 nM), shifted the amplitude distribution to the left without significantly diminishing the frequency of spontaneous events. Taken together, these results strengthen our hypothesis that phorbol ester increases spontaneous transmission by enhancing the opening of presynaptic voltage-dependent calcium channels, thereby augmenting transmitter release.

G.M. is a Lucille P. Markey Scholar and this work was supported by a grant from the Lucille P. Markey Charitable Trust.

K.D. Parfitt and D.V. Madson, Madison, Wisconsin activated the glial enzyme with significant response — curves from 10-7 to 10-3 M. No activation of the neuronal fractions isolated from rat brain and also in cultured astrocytes or neurons. The results clearly indicate that 5-HT selectively activates the glial enzyme with significant response curves from 10-7 to 10-3 M. No activation of the neuronal enzyme was observed at physiological 5-HT concentrations, Potassium activation curves and (3H) Ouabain binding in glial enriched fractions and cultured astrocytes, suggest that 5-HT may act by exposing more active sites of the enzyme. Our results suggest that regulation of glial Na+/K+-ATPase by a neurotransmitter may play an important role in maintaining the cationic balance for neuronal function.

The electrical stimulation of the median raphé nucleus (MRN) inhibits the spontaneous firing of medial prefrontal cortical (PFC) neurons, and this effect is likely mediated by 5-HT, raphe neurons (Manz et al., Brain Res., 524: 22-30, 1990). Biochemical and autoradiographic studies have shown that RP 62203, a novel napsiltamin derivative, is a potent and selective antagonist of 5-HT receptors. To further characterize the selective influence of RP 62203 on 5-HT neurotransmission in the PFC, we have studied the effect of i.p. administration of RP 62203 on 1) the 5-HT inhibitory responses induced by MRN stimulation and 2) the dopaminergic (DA) inhibitory responses elicited by stimulation of the ventral tegmental area (VTA). A single dose was administered and 1 PFC cell was tested in each of 10 ketamine-anesthetized rats. In 8 of the 10 PFC cells inhibited by both MRN (dur = 87.0 ± 5ms; lat = 10.4 ± 2ms) and VTA (dur = 114.0 ± 18ms; lat = 60.2 ± 2ms), RP 62203 blocked MRN-induced inhibitory responses in a dose-dependent and reversible fashion; in contrast, it failed to affect the inhibition elicited by VTA stimulation. These results show that RP 62203 selectively blocks 5-HT but not DA neurotransmission in the rat PFC and further support the involvement of 5-HT receptors in this effect.

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**594.3 INHIBITION OF PLATELET SEROTONIN UPTAKE BY PHORBOL ESTER: DOES PROGESTERONE CATION ACTIVATION DOWN-REGULATE THE SEROTONIN TRANSPORTER? G.M. Anderson*, W.C. Horne*, L.M. Hall*, B.A. Shaywitz, D.J. Cohen*. Child Study Center, Yale University School of Medicine, New Haven, CT 06510.**

Treatment of human platelets with 4-beta-12-o-tetradecanoylphorbol-13-acetate (beta-TPA), an activator of protein kinase C (PKC), resulted in a mean 20% decrease (N=16, p <.0001) in the Vm of serotonin (5-HT) uptake. A smaller effect on K m was also observed (mean 15% decrease, N=16, p <.0001). 4-Alpha-TPA was without effect on either the Vm or K m of a platelet 5-HT uptake. Treatment with the calcium ionophore A23187 produced a nonsignificant, non-specific decrease in Vm while dibutyryl cyclic AMP treatment had no effect. Phorbol ester (beta-TPA) did not cause a decrease in 

**594.4 COLOCALIZATION OF PROGESTIN RECEPTORS AND SEROTONIN IN RAPHE NEURONS. OF MACAQUE C. J. Bergh, Div. of Reproductive Biology and Behavior, Oregon Regional Primate Res. Ctr., Beaverton, OR 97006.**

Serotonin (SHT) neurons play a significant role in the regulation of several endocrine systems. Dysfunction of SHT neurons has also been implicated in various affective disorders, including depression. We have shown that progesterone (P) increases prolactin secretion in estrogen-primed monkeys, and that lactational prolactin receptors (PR). Since stimulation of SHT increases prolactin secretion, we questioned whether P could act on SHT neurons to increase prolactin secretion. Pituitary and mediobasal hypothalamic tissues were obtained at autopsy of a female Japanese macaque. SHT neurons were immunohistochemically identified in dorsal and ventral pontine raphe groups, as well as in a more caudal medullary raphe group in the macaque. SHT neurons were immunohistochemically identified in dorsal and ventral pontine raphe groups, as well as in a more caudal medullary raphe group in the macaque.

**593.1 PHORBOL ESTERS INCREASE THE FREQUENCY OF SPONTANEOUS EXCITATORY SYNAPTIC CURRENTS IN HIPPOCAMPUSS BY A PRESYNAPTIC, CALCIUM-DEPENDENT MECHANISM. K.D. Parfitt and D.V. Madson, Madison, WI.**

Using whole-cell voltage clamp techniques to study synaptic transmission in area CA1 of 500-600 thick hippocampal slices, we have found (Parfitt & Madson, Soc. Neurosci. Abstr. 1990) that phorbol esters are capable of potentiating stimulus-evoked synaptic transmission when 4-alpha-TPA was without effect on either the Vm or K m of a platelet 5-HT uptake. Treatment with the calcium ionophore A23187 produced a nonsignificant, non-specific decrease in Vm while dibutyryl cyclic AMP treatment had no effect. Phorbol ester (beta-TPA) did not cause a decrease in 

**593.2 DIFFERENTIAL MODULATION OF INHIBITORY TRANSMISSION IN THE HIPPOCAMPUSS BY CARRABACH, ADENOSINE, BACLOFEN, SEROTONIN AND NOREPINEPHRINE. G.A. Cohen, V.A. Does and D.V. Madson, Department of Molecular & Cellular Pharmacology and Communication Sciences, School of Medicine, University of Washington, Seattle, WA 98195 Carbach (CARR), baclofen (BACLO), serotonin (5-HT), norepinephrine (NE), and 2-adenosine (ADEN), all reduce the polymeric inhibitory postsynaptic potential (ipsi) in area CA1 of the rat hippocampus.**

Intracellular recording and whole cell patch clamping were used to monitor evoked ipsips and miniature ippss, respectively, in rat CA1 pyramidal neurons. We studied the effects of the listed neurotransmitters on evoked activity at different concentrations: 5-HT, NE, or ADEN (all 10uM) had an effect on the evoked polymeric ipsi. 5-HT, NE, or ADEN (all 10uM) had an effect on the evoked polymeric ipsi. 5-HT, NE, or ADEN (all 10uM) had no effect on the monosynaptic evoked ipsi. 5-HT (10uM) reduced the frequency of spontaneous ippss more than 50%, while the overall shape of their amplitude distribution was not changed. Baclo (10uM) caused a 10% reduction in frequency, and had no effect at 1muM. 5-HT, NE, or ADEN (10uM) had no effect on spontaneous miniature ippss.

5-HT, NE, and ADEN appear to inhibit interneurons at a somatic location or by inhibiting excitatory synaptic activation of interneurons. At low concentrations BACLO does not significantly decrease spontaneous GABA release but may act at the interneuron terminal (e.g., by decreasing calcium currents). CARB directly reduces GABA release from interneuronal synaptic terminals.

D.V.A. is a Lucille P. Markey Scholar and this work was supported by a grant from the Lucille P. Markey Charitable Trust. G.A.C. is a Howard Hughes Medical Institute Predoctoral Fellow. T.A.D. is a NIH Predoctoral Fellow.

**SEROTONIN III**
with no homology to tyrosine or phenylalanine hydroxylase. The peptides were the C-terminus. Support: NSF grant 88-12892 and Natl Down Syndrome Soc.

Both antibodies at 1/100-1/2000 revealed staining with WOH-66 showed specific staining in all the classical raphe nuclei

pineal and midbrain raphe tissue from adult rats were run on 12.5% SDS-PAGE characterized by using immunohistochemical staining of rat brain sections. The band (MW 47,800) for the midbrain raphe. The specificity was further

positively stained. No staining was seen in the locus coeruleus, substantia nigra

that two weeks of treatment with either drug did not alter 5-HT₄ receptors in the dorsal raphe nucleus. We have examined the effects of repeated treatment with two 5-HT uptake inhibitors used as antidepressants (fluoxetine and clomipramine, 10 and 20 mg/kg daily, respectively) on its density using autoradiography with 3H-8-OH-DPAT as ligand. Preliminary results showed that alpha-MTrp was injected. In each animal, it was possible to compare the rate of transport to the non-lesioned side, as a function of the distance from the dorsal/medial raphe

radioactivity ratio in the MFB and that found in the hypothalamus on the

rat serotonin (5-HT₁A) receptor. We also conclude that anterogradely transported 5-HT has some physiological relevance, as there would otherwise be no need for presynaptic control of the rate and amount of 5-HT transported from the cell bodies towards terminals. The work described here was supported in part by a grant from the MRC (MA-10023).

**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991**
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991

594.1

Previous studies from this laboratory revealed that in the rat, orally administered D-Fenfluramine for short periods (up to 4 days) produced short-lived and reversible immunocytochemical changes in neocortical 5HT innervation. (Kalia, M., Brain Res. 1991) In this report additional questions have been addressed: How long does the depletion of 5HT from the neocortex last following cessation of treatment? Is this effect reversible? We selected two oral doses, (8mg/kg/day and 16 mg/kg/day) which were administered for 21 & 30 days. The animals were examined at 3 different post-treatment time points: 18 hrs, 30 days and 60 days. Immunocytochemical methods, identical with those described previously were used to evaluate the brains postmortem. A predictable reduction in immunocytochemical staining of neocortical 5HT nerve networks was seen 18 hrs following cessation of treatment. The 5HT immunocytochemical staining remaining 30 and 60 days post-treatment was indistinguishable from pair-fed controls. When the results of 18 hour post-treatment were compared with existing data from 4 day treated animals, we found qualitative differences. Chronic treatment with D-Fenfluramine produced fewer "thick" immunoreactive 5HT fibers and the fine fibers were very distinct and finely beaded. These results confirm our hypothesis that the predictable reduction in neocortical 5HT immunoreactivity is a function of transient depletion of 5HT and is not associated with any long-term change in the 5HT innervation of this region.

594.2
PATTERNS OF NEURAL ACTIVITY THAT DETERMINE THE CYCLOPHERYSIS OF C. ELEGANS. Dorothy WC. Liu and James Thomas*, Department of Genetics University of Washington, Seattle, WA 98195.

Defecation is a periodic behavior in C. elegans, occurring about every 45 seconds in well-fed, adult wild-type worms. The cycle periodicity (CP) may be under the control of a neuronal pattern generator. Wild-type worms will stop defecating in the absence of food and CP is correlated with the rate at which the pharynx pumps; pumping defective mutants defecate less often. Excessive pumping in normal mutants yields only one mutation in one gene (dec-1) which caused lengthening of the defecation CP (Thomas, Genetics 124:855-872, 1990). We reasoned that Con screens might fail to reveal many mutants with long cycles and should miss mutants with shorter than normal CP.

To isolate short and long-cycle mutants we undertook a direct screen for animals with altered CP, after EMS mutagenesis. CP was timed in individual animals, and a mutant candidate was picked: 1) if it repeated the behavior twice or more in one minute, 2) if it repeated the behavior less often than once per minute.

The screen has led to the identification of three classes of mutations, which affect CP by: causing: 1) long cycles, 2)short cycles, and 3) variable CP. Most of the mutations we have isolated are of the first class. An example is the sa92 mutation, which causes an average period of 100 seconds. We have isolated one mutation in the second class, sa92, which causes a CP of about 30 seconds. Finally we have isolated a mutation (sda96), which causes animals to have a variable CP ranging from 10-150 seconds. Thus far, none of the mutations map to the same loci and we are carrying out epistasis analysis.

We are currently examining the effects of these mutations on other rhythmic behaviors such as egg-laying and locomotion. In order to identify the cells which are responsible for controlling periodic behavior, we are using a microscope-focused laser to kill neurons in wild-type worms.

594.3
MODULATION OF IDENTIFIED STOMATOGLASTIC NEURONS IN PRIMARY CELL CULTURE. O.G. Turjman and E. Marder, Dept. of Biology, Brandeis Univ., Waltham, MA 02254.

We have begun to use current and voltage clamp techniques to study the properties of identified stomatogastric neurons in primary cell culture. Gastric mill and pyloric neurons from the stomatogastric ganglion of Panulirus interruptus are identified electrophysiologically, ganglia are enzymatically treated, neurons are dissociated by aspiration into micropipettes, and plated onto uncultured Nunc culture dishes in defined medium. Over the course of several days these neurons regenerate neurites, and regain the ability to produce action potentials, to oscillate in the presence of TEA, and to respond to neuromodulators. The PD neuron, a likely target of octopamine in situ, is modulated by octopamine in culture: octopamine acts in part by reducing the magnitude of an A-type current in this neuron. The PD neuron in culture is excited by the muscarinic agonist pilocarpine, inhibited by dopamine, and is not affected by serotonin; these responses are similar to those exhibited by PD in situ. These preliminary results suggest that pyloric and gastric mill neurons are expressing the same complement of receptors in culture as in situ, and are retaining some of their physiological properties. Supported by NS-08971 to O.G.T. and BNS-9009251 to E.M.

595.1
MUTATIONS AFFECTING PERIODIC BEHAVIOR IN C. ELEGANS. Dorothy WC. Liu and James Thomas*, Department of Genetics University of Washington, Seattle, WA 98195.

Defecation is a periodic behavior in C. elegans, occurring about every 45 seconds in well-fed, adult wild-type worms. The cycle periodicity (CP) may be under the control of a neuronal pattern generator. Wild-type worms will stop defecating in the absence of food and CP is correlated with the rate at which the pharynx pumps; pumping defective mutants defecate less often. Excessive pumping in normal mutants yields only one mutation in one gene (dec-1) which caused lengthening of the defecation CP (Thomas, Genetics 124:855-872, 1990). We reasoned that Con screens might fail to reveal many mutants with long cycles and should miss mutants with shorter than normal CP.

To isolate short and long-cycle mutants we undertook a direct screen for animals with altered CP, after EMS mutagenesis. CP was timed in individual animals, and a mutant candidate was picked: 1) if it repeated the behavior twice or more in one minute, 2) if it repeated the behavior less often than once per minute.

The screen has led to the identification of three classes of mutations, which affect CP by: causing: 1) long cycles, 2) short cycles, and 3) variable CP. Most of the mutations we have isolated are of the first class. An example is the sa92 mutation, which causes an average period of 100 seconds. We have isolated one mutation in the second class, sa92, which causes a CP of about 30 seconds. Finally we have isolated a mutation (sda96), which causes animals to have a variable CP ranging from 10-150 seconds. Thus far, none of the mutations map to the same loci and we are carrying out epistasis analysis.

We are currently examining the effects of these mutations on other rhythmic behaviors such as egg-laying and locomotion. In order to identify the cells which are responsible for controlling periodic behavior, we are using a microscope-focused laser to kill neurons in wild-type worms.

595.2

In crayfish, the rhythmic activity of the swimmeret system is strongly modified by stimulating the second thoracic roots (STR). This stimulation increases the frequency of the swimmeret rhythm and the frequency of spikes within bursts, it also increases the total amplitude of membrane oscillations. We have isolated a set of certain swimmeret neurons. Sometimes, an increase in the intensity of stimulation inhibits the swimmeret rhythm.

Recently, we have found that stimulation of STR affects not only the swimmeret system but also the abdominal positioning and the walking systems. The STR effects are most of the time comparable to that of spontaneous activity in these systems often obtained during the same experiment. In the present study we have also been able to record intracellularly from a number of abdominal interneurons. Most of these interneurons respond to the stimulation of the STR with a long-lasting depolarization, while others with only a short-lasting membrane depolarization and/or EPSPs. These properties may explain the variety of the STR effects.

In conclusion, axons in the STR seems to have access to a wide range of abdominal interneurons, some with specific effects (i.e. activation or inhibition of the swimmeret rhythm), while others seems to have a multiple motor output properties. At least some of these interneurons seems to induce effects that are established slowly and are of long duration suggesting that the stimulation of the STR might involve command neurons. Finally, intracellular recording from the same interneuron in two different ganglia suggests that they are descending interneurons.

This work has been supported by an ERC grant ERB896198.

595.3
MECHANISMS SUBSERVING CONSTRUCTION OF A NOVEL MOTOR-PATTERN GENERATING NETWORK BY A MULTIACTION INTERNEURON. A. J. Simon , P. Moreaux and M. Mouilla. Lab. de Neurobiologie et Physiologie Compiltes, Univ. de Bordeaux I, CNRS, Arcachon, 33120 FRANCE.

We have recently reported that in the stomatogastric nervous system (STNS) of lobster (Homarus gammarus), the rhythmic discharge of an identified modulatory neuron (PS) is able to construct de novo the central pattern generator (CPG) for swallowing-like behavior from neurons belonging to other functional CPGs (Nature, 1991, in press). Thus, when PS is active, neurons otherwise operating independently as integral elements of different neuronal networks are reconfigured into an completely new functional circuit.

We report here on the cellular mechanisms underlying this network reconfiguration. A study of direct effects of PS on individual STNS neurons has revealed three major types of postsynaptic action. First, PS acts directly on many neurons via conventional short-term synapses that evoke discrete excitatory or inhibitory postsynaptic potentials (PSPs). Second, PS can exert long-term influences that far outlast the discharge of the interneuron itself. These modulatory influences involve the suppression of intrinsic bursting properties in certain STNS neurons, while in others, a sustained membrane depolarization is produced. Third, PS may act on the same target neuron via both short and long-term influences, giving rise to transient PSPs in combination with the modulation of bursting properties.

Our results show how a neuronal network can be dynamically assembled from neurons of disparate origin by the multiple actions of a single interneuron; conventional synapses serve to coordinate different individuals to the cell own firing pattern, while modulatory influences appear to dictate both functional membership in the new circuit and the duration of its existence.
Using chemotaxis as a model behavior, we trained neural networks in
Activation of the sensory units was determined by the position of the
The network had two sensory units and two motor units. The velocity
biological network. However, in most systems, more is known about
trained successfully using recurrent backpropagation to find and remain
but little is known about events occurring locally in these
This feed-back must occur locally, in the STG neuropil,
when fictive mastication is induced by stimulation of the sensorimotor cortex
rumbo of neurons that did not receive SLEIs was due to phasic
inhibitor cells, PIR is induced by 5-HT, is prolonged, and is of low
induces or enhances postinhibitory rebound (PIR) in swim motor neurons
also in oscillator interneurons 28, 208, and 115 of the leech swim circuit.
As phase shifts occur, it scales the ratios of the current input signal strengths at each synaptic
to receiving local synaptic feed-back from these networks.
the model to predict the contribution of real neurons to observed
connections. Additional constraints on the neural circuit should allow
axon for long distances to arborize in the STG neuropil,
Local Interactions of Descending Inputs with Rhythmically
compilation of data from both experiments and simulations. A neural network model was
identified to the playing of a fragment of its recorded piece or to the playing of the
the experimental data, and the results from the network model were compared.
the interactions and activity of motor neurons, which have reciprocally
The model is non-Hebbian in that the reinforcement of individual synapses
case activity from tonic to rhythmic. These results suggest that
endogenous, spontaneous descending activity may also be
when stimulation of the contralateral SMC was 8.3 ms (n = 9) compared to 9.6 ms
other 2 fired to stimulation of either SMC. The mean of the modal latencies to
phasic inhibition also
occurred. Supported by the Canadian MRC.
recurrent swim episodes in isolated leech ventral nerve cords (Willard, J.
this correlation is found, the model determines that the ratio of each synaptic pair is
cooperation among the different levels of the network. The model can
thresholding is applied at each neuronal level to reduce noise.
cells to the program driven by CBI-2 and are monosynaptically connected to it. B17
is a postsynaptic cell that receives powerful, slow excitatory input from CBI-2
B17 itself drives a motor program, 5(6)-Carboxyfluorescin dye fills indicate that B17
biasing axons in the buccal and cerebral ganglia and in buccal nerves 3.
B19 is a presynaptic cell that evokes a complex, slow inhibitory and excitatory
endogenous, spontaneous descending activity may also be
When fictive mastication is induced by stimulation of the sensorimotor cortex
feeding involve interganglionic CPG neurons that exhibit feedforward and
The pyloric network also affected
the pyloric network had two sensory units and two motor units. The pyloric
and chemically-mediated PSPs from
gastric mill neuron LG. The pyloric network also affected
by 5-HT and is prolonged. Serotonin increases the amplitude and duration of
neuronal activity in the isolated leech swim circuit. It is often observed that
5-HT. Cells were injected with constant, hyperpolarizing current (1
application of micromolar concentrations of serotonin (5-HT) elicits
A neural network model was used to simulate the behavior and network activity of the
because it is related to the input to the network. The model is trained using
neural network for the reproduction of input and output neurons in the biological network.
Some properties of neural circuits can be predicted by training a network model to
reproduction of the activity of input and output neurons in the crustacean
The pyloric and gastric mill networks in the crustacean
be simulated by a simple computer model.
neural network to reproduce the activity of input and output neurons in the
endogenous, spontaneous descending activity may also be
return trigger is also used to stop the process. The process simply records the activation ratios of
each synaptic pair. Several physiological, biochemical, and molecular biological
mechanisms are envisioned which could perform the operations of this model.
the network model with three neurons of 123 levels and 12
synaptic pairs per level recording through the musculature of pieces of up to 73
inhibitory connections with oscillator network cells, may be critical to the
neuron is divided into a tonically stimulated SNAX 1, thereby transforming its
and the other two neurons remained nearly inactive. Each neuron also responds to the
neurons, which are electrotonically close to the STG neuropil, enabling
axons for long distances to arborize in the STG neuropil,
neuron to the program driven by CBI-2 and are monosynaptically connected to it.
B17
of input and output neurons in the isolated crustacean ganglia. This
input to the program driven by CBI-2 and are monosynaptically connected to it. B17
initially reconfigured.
END OF ABSTRACTS, VOLUME 17, 1991
596.1 SYNCHRONIZATION OF INTERNEURONS IN THE HIPPOCAMPUS. H.B. Michelson and R.K.S. Wong. Dept. of Pharmacology, State Univ. of New York, Health Science Center, Brooklyn, N.Y. 11203.

Synchronized inhibitory post-synaptic potentials (IPSPs) can be recorded in guinea pig hippocampal pyramidal and granule cells in the absence of glutamate-mediated excitation. These synchronized IPSPs are recorded in the slice in 4-aminoypyridine (4-AP, 75 μM), CNQX (10 μM) and APV (10 μM). Deafferentation from inhibitory interneurons confirmed that a population of these cells were firing simultaneously to generate the synchronized IPSPs in principal cells.

Picrotoxin (50 μM) initially suppressed the rhythmic synchronized IPSP in principal cells. A second, different hyperpolarizing event subsequently emerged. This synchronized event had a slower time-to-peak (30.3 ± 15.4 ms), was blocked by salicylate, and occurred at a lower frequency than the picrotoxin-sensitive event.

Picrotoxin (50 μM) blocked the burst events in a subpopulation of interneurons, indicating that excitation between this group of interneurons was mediated by GABA. Some interneurons continued to burst in the presence of picrotoxin. These bursts occurred simultaneously with the slower synchronized IPSP in principal cells. We conclude that GABAergic interneurons can become synchronized by at least two non-glutamate dependent processes: excitation mediated by the GABA receptor, and a process independent of amino acid transmission. (Supported by N.I.H. and the American Epilepsy Society)

596.2 EPILEPTOGENESIS FOLLOWING CORtical INJURY. D.A. Prince and G. F. Tsirka. Dept. of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305.

We developed an in vitro model of chronic epileptogenesis by taking advantage of the hyperexcitability and epileptogenic known to develop in chronically isolated in vitro. Partially isolated neocortical islands were prepared in F12-P24 rats 17-122 days later, slices were prepared through the previous lesion. Orthodromic stimuli were increased but not control condition, and long and variable latency field potentials that had the characteristics of epileptiform events. Discharges were maximal in layer 2-3 and propagated into deeper layers and across the slice. Bath application of APV blocked the long latency discharges. Intracellular recordings showed that epileptiform field potentials were accompanied by polyphasic EFSPOs without prominent inhibitory events. In both postnatally and adult male animals bicuculline- or pentavyl-pyridine- treated rats showed GABAergic interneurons remarkable elaboration of recurrent axon collaterals extending to supragranular layers. Some neurons showed the typical picture of recurrent axonal sprouting described in immature isolated cortex by Cajal and Purpura and Housepian (Exp. Neurol. 4:377, 1961). These preliminary results suggest that chronically undercut cortex studied in vitro is an excellent model for examination of development of chronic epileptogenesis after brain injury. Alterations in pyramidal cell membrane properties, development of new excitatory connectivity, and depression in inhibitory synaptic electromography may play a role in the abnormal discharges generated in vivo. Supported by NIH grants NS06477 and NS12151 from the NINDS, and a Pinney Postdoctoral Fellowship.

596.3 FORNIX LESIONS ALTER EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL SLICES. P.A. Redgrave. Soc. of Neurophysiol., Dept. of Neurology, Baylor Coll. of Med., Houston, TX 77030.

Chronic (10-90 day) unilateral fornix lesions in rats result in hippocampal slices that have properties for epileptiform activity. We assayed for amino acid transmission. To assess effects of fornix lesions on local CA3 synaptic circuitry, epileptiform discharges were produced by 50 μM picrotoxin (picro) or by 8.5 mM [K+]o saline and recorded extracellularly. The duration and frequency of occurrence of epileptiform discharges in slices from control animals and from hippocampal ipsi- and contralateral to the lesion were compared. In picro, no difference in the rate of discharges in the three groups was observed, but the duration of the discharges was significantly (P<0.05) longer in slices ipsi- and contralateral to the lesion compared to control slices (120±7.5 vs. 116±6.7, and 93±5±3.0 vs. 94±5±0.0, respectively). In 8.5 mM [K+]o saline, discharges from slices ipsi- to the lesion were longer than those from contralateral slices (80±6.3±7 vs. 63±6±3.3 ms) and occurred at a faster rate than those from control animals (0.68±0.6 vs. 0.45±0.0 Hz). The effects of the NMDA antagonist APV (50 μM) on convulsant induced bursting was studied. On average, APV slowed the rate of picro induced bursting in slices from lesioned animals by greater than 50%, but did not affect those from control animals. APV tended to shorten burst duration, but this did not reach statistical significance. In 8.5 mM [K+]o, saline, APV slowed significantly the epileptiform discharges in slices ipsilateral to the lesion, but did not significantly change the rate in slices taken from control or contralateral hippocampus. The duration of high [K+]o bursts was shortened by APV in all three groups. These results suggest that deafferentation of the hippocampus by fornix lesions altered the response of the CA3 region to convulsants and enhanced the NMDA-mediated component of epileptiform activity. (Supported by NIH grant NS35580 and the Klingenstein Fund.)


Kindling is a model of epilepsy and neuronal plasticity in which repeated electrical stimulation of certain CNS structures gradually leads to development of generalized clonic convulsions. Evidence suggests that PKC plays an important role in various forms of neuronal plasticity, including LTP in the hippocampus and activity-dependent cortical development. To investigate the possible role of PKC in kindling-induced epileptogenesis, we determined the effect of amygdala kindling on PKC activity in various regions of rat brain. We first assayed for changes in kinase activity by assaying post-hoc phosphorylation of endogenous proteins. We observed that kindled markedly elevated basal (Ca²⁺-) and Ca²⁺-stimulated phosphorylation of an endogenous protein in dentate gyrus which we have termed P17. Increased phosphorylation of P17 was observed 2 hours after the last stimulus-induced seizure and did not occur in limbic regions other than the dentate gyrus. Phosphorylation was decreased in dentate gyrus 24 hours after seizure. The amount of P17 protein remained constant. Assay of the amount of P17 protein revealed a transient increase in P17 protein 2 hours after the last seizure. Addition of a competitive peptide inhibitor (PKCpK 36) to in vitro stimulation in an exogenous substrate, also showed a transient increase in basilar and Ca²⁺-stimulated activity in kindled dentate gyrus, 2 hours after kindling stimulation. These results document a kindling-associated alteration in PKC activity. Interestingly, these observations also suggest that there is a coordinate up-regulation of PKC and the level of one of its substrates in the kindled dentate gyrus.


Seizures are invariably followed by profound behavioral depression and by enhanced inhibitory post-synaptic potentials, but the responsible mechanisms are unknown. We investigated GABA metabolism in the substantia nigra (SN) of rats kindled by stimulation of medial septum. SN GABA concentration was 93±11 μmol/g prot in untreated controls, 87±1 μmol/g prot. in naive rats subjected to septal stimulation, and 107±1 μmol/g prot. in kindled unstimulated rats, and 138±11 μmol/g prot in kindled rats stimulated to a stage 5 seizure 60 minutes before SN tissue was sampled (Gp 4).

The corresponding rate of GABA synthesis in SN of these 4 groups of rats was respectively 57±8, 69±14, 68±7, and 116±20 μmol/g prot/hr. Only the GABA concentration in SN of Gp 4 was significantly different from controls. The increase of GABA concentration and synthesis in SN was observed post-ictally; 5 to 60 min after seizure activity had ceased, the rate of GABA synthesis in SN was 160 μmol/g prot/hr compared to 68 μmol/g prot/hr pre-ictally. These changes may provide a potential explanation for the postictal depression and enhancement of inhibitory processes. Supported in part by funds from the V.A. Research Service and by grant NS13515 from NINDS.


The kindling model of epilepsy is associated with long-lasting changes in Type II Calmodulin kinase (CaMKII) activity and immunoreactivity. In order to determine the mechanism of these alterations, we measured gene expression of CaMKII using in situ hybridization in septally kindled rat brains and pons controls using a 35 S labelled 83 base pair probe for the CaMKII beta subunit. We found the mRNA concentration in the hippocampus and other limbic structures in a similar pattern of staining observed immunohistochemical. Kindling resulted in significant decrease in hippocampal CaMKII mRNA: 30% in CA1, 34% in CA2, 35% in CA3, 41% in CA4, and 29% in the dentate gyrus. Hybridization was also decreased by 21% in the cortical cortex but not in the lateral septum. These changes are similar in distribution and direction as those measured immunohistochemically. These data suggest that altered CaMKII activity and immunoreactivity associated with kindling reflect long-lasting alterations in gene expression of this synaptic protein and provides further evidence for its possible importance in the kindling phenomenon.
596.7 KINDLING REDUCES THE SENSITIVITY OF PRE-BUT NOT PONTOSYNAPTIC GABA<sub>B</sub> RECEPTORS IN THE BASOLATERAL AMYGDALA (BLA): L.K.Aspaglidi, D.G. Rainnie, & P. Shinnick-Gallagher, Deps. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77550.

KINDLING results in an enhancement of excitation and a loss of inhibitory responses elicited by stimulating the stria terminals (ST) in BLA neurons. Since excitatory transmission through GABA<sub>B</sub> receptors on excitatory terminals, we tested whether alterations in pre-synaptic GABA<sub>B</sub> receptors contributes to the kindling-induced enhancement in excitatory responses using standard intracranial injection of baclofen (0.1-500nM) depressed ST evoked synaptic responses in control (C) and kindled (K) BLA neurons. The EC<sub>50</sub> for this action of baclofen was shifted 100-fold from 5mM in control to 300mM in kindled neurons (C=29; K=23). When GABA<sub>A</sub> and post synaptic GABA<sub>B</sub> receptors were blocked with bicuculline (nic; 30mM), CNQX (10mM) to block the bicuculline-induced bursting, the intracerebral GTP-like <sub>75</sub> (10mM), baclofen (20mM) reduced the evoked slow EPSPS to a greater extent in control than in kindled neurons (C:5±2.9% n=3; K:15±5.6% n=4; p<0.01). Baclofen (20mM) had no effect on NMDA (n=3) or AMPA (n=3) responses recorded with a GTP-Y-S filled electrode. The EC<sub>50</sub> for post synaptic GABA<sub>B</sub> receptors was 1μM in control and in kindled neurons (C=31; K=21). 2-OH-Saclofen (2-OH-sac, 100nM) blocked baclofen-induced hyperpolarizations in control (n=7) and kindled (n=10) neurons; however, in 2-OH-sac baclofen (100mM) still reduced EPSP amplitude by 45.7±3.4% in control and by 28±5.6% in kindled neurons.

These data suggest that the kindling-induced changes in baclofen sensitivity pre-synaptically cannot be due to a loss of GABA<sub>A</sub> inhibition, or an effect on post synaptic glutamate or GABA<sub>B</sub> receptors. The differential change in the sensitivity of GABA<sub>B</sub> receptors, and the response to 2-OH-sac may indicate distinct subpopulations of GABA<sub>B</sub> receptors whose long-lasting response to the kindling stimulus is different. Supp. by NS 24643.

596.9 ADENOSINE KINASE AND ADENOSINE DEAMINASE INHIBITION IN RAT PREPIRIFORM CORTEX CONFERS PROTECTION AGAINST BICUCULLINE METHOXYDE-INDUCED SEIZURES Q. Zhang and T.F. Murray, College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Our previous studies have demonstrated the profound effects of the adenosine receptor antagonist 8-p-sulfonylphenyltheophylline and the anticonvulsant effects of the adenosine transport blocker dilaizep, suggesting that adenosine may function as an endogenous antiepileptic substance in vivo. To further support the hypothesis that adenosine plays a role in the regulation of seizures, the aim of this study is to examine the influence of an adenosine kinase inhibitor and an adenosine deaminase inhibitor on bicuculline methyloide-induced seizures in this brain area. All drug solubilized into the PFC. We found that focal injection of the adenosine kinase inhibitor, 5'-amino-5'-deoxyadenosine (ADADO), protected rats from BMEI seizures in a dose-dependent fashion (ED<sub>50</sub>=2.6±0.8 nmol). A dose of 68.4 nmol of ADADO (100nM) still reduced EPSP amplitude by 45.7±3.4% in control and by 28±5.6% in kindled neurons. These data suggest that the adenosine kinase inhibitor, 5'-amino-5'-deoxyadenosine (ADADO), protected rats from BMEI seizures in a dose-dependent fashion (ED<sub>50</sub>=2.6±0.8 nmol). A dose of 68.4 nmol of ADADO (100nM) still reduced EPSP amplitude by 45.7±3.4% in control and by 28±5.6% in kindled neurons.

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The present study determined the effects of the non-competitive NMDA antagonist MK-801 on behavior in the conditioned suppression of drinking (CSD) paradigm, a repeated measures conflict task. In daily 10-minute sessions, water-restricted rats drank from a tube which was occasionally electrified (0.25 mA shocks signaled by a tone). Trained subjects (4 weeks of CSD testing) exhibited stable baselines for both punished (41 ± 5 [Mean ± SEM] shocks received/session and unpunished (14.0 ± 0.7 ml/session) responding. Phenobarbital (5 - 40 mg/kg, IP) or chloridiazepoxide (2.5 - 20 mg/kg, IP) administration with a short (10- or 30-minute) pre-treatment resulted in a dose-dependent increase in punished responding, whereas (+) MK-801 did not increase punished responding when administered using a 10-minute or 4-hour pre-treatment. However, at a 24-hour pre-treatment (+) MK-801 (0.01 - 0.4 mg/kg, IP) produced a dramatic and dose-dependent increase in punished responding. The "inactive" isomer (-) MK-801 did not produce an anxiolytic-like effect in the CSD paradigm at any pretreatment interval. The anti-convulsant (+) MK-801 also may possess anxiolytic effects in humans. (Supported in part by MH 47181.)

597.2 EFFECTS OF ANTI-SEIZURE AGENTS ON CONFLICT BEHAVIOR IN THE RAT. T.J. Hill, D.J. Fontana and R.L. Commissaris. Dept. of Pharmaceutical Sci., College of Pharmacy, Wayne State University, Detroit, MI 48202.

Traditional anti-seizure agents recently have been used in psychiatry for the treatment of a number of disorders, including anxiety disorders. The present study examined the effects of acute treatment (10- to 120-minute pretreatment) with several anti-seizure agents in the conditioned suppression of drinking (CSD) conflict paradigm, an animal model for the study of "anxiety" and anti-anxiety agents. The agents tested were diazepam (DZ), phenobarbital (PHB), carbamazepine (CBZ), phenytoin (DPR), valproate (VPA), and ethosuximide (ESI). Consistent with their anxiolytic effects in man, DZ (1.25-20 mg/kg) and PHB (5-40 mg/kg) produced dose-dependent and robust (maximal effect 50 - 70 shocks above control) increases in punished responding. CBZ (2.5 - 40 mg/kg) and VPA (50 - 200 mg/kg) produced dose-dependent increases in punished responding, but the maximal effect was less than that seen with PHB or DZ. Neither ESI (25 - 100 mg/kg) nor DPH (0.6 - 20 mg/kg) increased punished responding. Thus, not all anti-seizure agents exert anxiolytic-like effects on conflict behavior. (Supported in part by MH 47181.)

597.3 BEHAVIORAL EFFECTS OF INTERACTIONS AMONG D1 AND D2 Dopamine Receptor Agonists and Antagonists. T. L. Katz and Michael J. Lovenberg. Psychology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224, U.S.A.

The effects on schedule-controlled operant behavior of the D1 receptor agonist, SKF 38393, and the D2 agonist, quinpirole, were assessed alone and in combination with selective dopamine-receptor antagonists. Squirrel monkeys (Saimiri sciureus) were trained to press a response key under fixed-interval and fixed-ratio schedules of food reinforcement. Quinpirole increased rates of responding under the fixed-interval schedule at doses of 0.1 - 1.0 mg/kg that decreased rates of responding under the fixed-ratio schedule. Under the fixed-interval schedule, the D2 agonist, quinpirole (0.003 - 0.006 mg/kg) and to less of a degree haloperidol (0.003 - 0.01 mg/kg), and the D1 agonist, SCH 23390 (0.03 mg/kg), shifted the quinpirole dose-effect curve to the right. At the highest doses of the agonists, the maximal effects of quinpirole were decreased, however, only the D2 antagonist, spiperone, antagonized the effects of quinpirole under the fixed-ratio schedule. The D1 agonist, SKF 38393, dose-dependently (1.0 - 10.0 mg/kg) decreased rates of responding. These schedules were not antagonized by any doses studied of either spiperone (0.003 mg/kg) or SCH 23390 (0.003 - 0.3 mg/kg). SCH 23390 produced a dose-related enhancement of the effects of SKF 38393.

597.4 DIFFERENTIAL EFFECTS OF D, AND D, ANTAGONISTS IN COMBINATION WITH THE D AGONIST (+)-PHNO IN SQUIRREL MONKEYS. Sharon Rosenweig-Lipton, Sara John* and Jack Bergman. Harvard Medical School/NERPRC, Southboro, MA.

Behavioral effects of the dopamine D1-selective agonist (+)-PHNO were determined alone and after pretreatment with the D1 antagonist eticlopride, the D2-selective agonist SCH 39166, and the nonselective dopamine agonist clonazepam in squirrel monkeys responding under a 30-minute fixed-ratio schedule of stimulus-shock termination. When administered cumulatively, (+)-PHNO (0.001 - 0.3 mg/kg) produced dose-dependent decreases in rates of responding, with ED50 values ranging from 0.002 to 0.014 mg/kg for individual monkeys. Etioclopride (0.001 - 0.006 mg/kg) produced graded rightward shifts in the dose-response curve for (+)-PHNO; ED50 values for (+)-PHNO were increased by as much as 30-fold for individual monkeys. In contrast, SCH 39166 did not antagonize the behavioral effects of (+)-PHNO at any dose. Instead, increasing doses of SCH 39166 (0.01 - 0.1 mg/kg) produced leftward and downward shifts in the dose-response curve for (+)-PHNO. Clonazepam (0.5 mg/kg) also resulted in graded leftward and downward shifts in the dose-response curve for (+)-PHNO. These results show that the rate-decreasing effects of (+)-PHNO can be surmountably antagonized by the D2 antagonist eticlopride but are modified differently by the D1 antagonist SCH 39166 and the atypical neuroleptic clonazepam. (Supported by USPHS grants DA03774, DA00499, MH07658, and RR00168)


MDMA profoundly depletes serotonin (5-HT) in nerve terminals in primate brain following several repeated doses. These findings were confirmed in adult male monkeys (n=5) to determine the effects of such 5-HT depletions on complex brain functions including learning, short-term memory and attention, time perception, color and position discrimination, and work to perform for food. Daily (27) performance in a multiple schedule OTB was used to model these functions with response rate, accuracy, and percent task completed serving as indicators of functional ability. Each dose of MDMA (0.0 mg/kg), 0.1, 0.3, 1.0, 3.0, 5.6, 10.0 and 20.0 mg/kg was given intramuscularly twice daily for 14 consecutive days in ascending order. Although there were clear acute effects of MDMA (generally at > 1.0 mg/kg) to disrupt performance of all tasks, significant decreases in performance were not demonstrated. OTB performance generally returned to predrug values a few weeks after chronic treatment ended, when 5-HT systems are presumably severely depleted. Thus, 5-HT systems would not appear to be of fundamental importance for the performance for these specific brain functions.

597.6 THE LACK OF SEDATIVE PROPERTIES OF CI-988, A SELECTIVE CCK, RECEPTOR ANTAGONIST. Lakhbir Singh, Ryszard J. Orzeszko and Geoffrey N. Woodruff. Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB, U.K.

Previously it has been reported that the selective CCK receptor antagonist CI-988 (previously PD 134308) produces anxiolytic-like action in a number of animal models of anxiety. Singh et al., 1990, Singh et al., 1991, have now show that CI-988 does not induce sedation/anxiety or potentiate the action of CNS depressants.

Chloridiazepoxide (CDP) or CI-988 were administered i.p. 40 min before test to male TO mice (25-30g). CDP dose-dependently (1-30 mg/kg, i.p.) impaired rotorod performance and increased sleeping time induced by sodium pentobarbital (60 mg/kg, i.p.) with minimum effective dose (MED) of 10 mg/kg. CI-988 also potentiated the ability of ethanol to impair rotorod performance. In contrast, CI-988 (up to 30 mg/kg which is 300-3000 fold higher than the anxiolytic dose was found to be inactive in all these tests.

Thus, it is suggested that CI-988 should have a better side-effect profile in man than the benzodiazepines.


598.1 SPROUTING IN THE NEUROMUSCULAR SYSTEM: EARLY SIGNALING BY IGFI FROM INFECTED MUSCLE FIBERS. C. C. Knoll, P. A. and Schmidtke, C. D. Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel.

Upon lesion- or toxin-induced paralysis processes in the inactive muscle fibers and in muscle containing nerve fibers. Among these is the local stimulation of nerve growth. Insulin-like growth factors (IGFs) can induce neurite growth in vitro, and IGFI mRNA in mice is up-regulated with the presence of growth inducing activity in fast skeletal muscle (Shan, PNAS (1998) 95:2899-2902). We are therefore investigating the possibility of IGFs in neuropeptide regeneration, including intramuscular regrowth (Davenport & Bulloch, MB (1991) 13:1207-1317; 3.) in the adult rat, paralysis leads to rapid (detectable after 15s) and transient (peak at about 48h) induction of IGF-I mRNA in muscle fibers (in situ hybridization data; DIG-alkaline phosphatase method), followed by a slower and persistent increase of IGFI and IGFI mRNA in muscle fibers and in muscle interstitial cells. For comparison, increased in muscle fiber N-CAM mRNA was detectable 3d after paralysis; 4.) adult rat motoneurons in vitro express IGFI-receptor mRNA (method as above). Since elevated IGFI levels are sufficient to trigger sprouting and intramuscular cell proliferation, and since muscle fiber IGFI mRNA levels are rapidly elevated upon paralysis, IGFI is likely to initiate regenerative responses in paralyzed skeletal muscle, possibly including a direct stimulation of nerve sprouting.


We tested the hypothesis that insulin-like growth factors (IGFs) and nerve growth factor (NGF) can all increase the expression of genes encoding major structural proteins of axons, particularly 68 and 170 kDa neurofilament (NF) genes, in human neuroblastoma SH-SY5Y cells. We also studied whether more than one transcriptional site for a single gene might be involved.

Insulin, IGFI, and IGF-II increased 68 and 170 kDa NF mRNA content relative to total RNA, poly(A)+ RNA, and histone mRNA during nerve outgrowth. Insulin and IGF-I had little effect on stability of NF mRNAs, whereas insulin increased transcription of 68 and 170 kDa NF mRNAs. Similar effects are reported for NGF (Lindenbaum et al., 1988). In contrast, our previous studies showed that insulin, IGFI, and IGFII all stimulate α- and β-tubulin mRNAs, but have no detectable effect on transcription. We conclude that insulin, IGF-I, and IGFII stimulate NF gene expression, and that insulin, IGF-I, and NGF can activate two branches of a common biochemical pathway regulating neurite outgrowth. (Supported by NIH grants NS24327 and NS24606.)

598.3 SEPTAL DENERVATION OF THE HIPPOCAMPUS IS REQUIRED FOR SYMPATHETIC AXON INGROWTH. B. N. Safiullah and R. A. Constable. Department of Neurology, University of Cincinnati, Cincinnati, OH 45267.

Sympathetic sprouting into the rat hippocampal formation (HF) after a medial septal lesion (MSL) may occur as a result of accumulation of NGF. Intraventricular infusion of NGF in the shaker, does not elicit sympathetic-hippocampal sprouting even though there is proliferation of sympathetic fibers (Brain Res. 492: 245). Furthermore, NGF infusion in the presence of a MSL results in reduced sympathetic-hippocampal sprouting, perhaps due to competition with periarterial collateral NGF mRNA (Brain Res. 525: 111). We tested whether intraventricular NGF infusion would promote sympathetic sprouting into the HF from autogenous superior cervical ganglion transplant. In the presence or absence of a MSL. Female SD rats received 2 week infusions of NGF beginning at the time of transplantation of the SCG to the HF. Four rats received a MSL, six did not. Animals were killed at 2 (n=3) and 4 (n=8) weeks after surgery and the brains were examined for sympathetic ingrowth (SPG method), septal denervation (ACE histochemistry) and NGF content (ELISA). Although extensive ingrowth was found in septal-denervated regions of the HF, an ingrowth was observed in animals without evidence of septal denervation (n=4). Furthermore, in animals where there was partial septal denervation (n=3), sympathetic axons were found only in denervated areas. In NGF-infused animals, contralateral septal denervation and hypervascularization of the internal carotid artery occurred. In 7 animals with transplants and NGF infusion, fibers extended from the transplants through the fibromuscle and corpus callosum to the ventricle where the cingulum was placed. These results indicate that NGF infusion stimulates sympathetic fiber outgrowth from transplantated ganglia but does not permit sympathetic sprouting in the HF in the presence of septal-hippocampal fibers. (Supported by NIH NS-17131.)

598.4 STAUROSPORINE NEUROTOPTIC EFFECTS IN PC12 CELLS ARE INDEPENDENT OF PROTEIN KINASE C. D. Hansen*, R. Lester*, Y. Matsuda* and P. Lazarovici*. "Dept. of Pharmacology, Hebrew University, Jerusalem, Israel, "Dept. of Membrane Biochemistry, Weizmann Institute, Rehovot, Israel and "Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan.

Nerve growth factor (NGF) is a polypeptide orchestrating the number of nerve growth inducing activity, including differentiation and survival. In PC12 cells, NGF induces differentiation expressed by neurite-outgrowth and neuronal network formation.

We have found that Stauoropine (ST), an alkaloid known to inhibit Protein Kinase C (PKC) in vitro, induces neurite outgrowth in PC12 cells within 6 hours at non toxic, 10-30nM, concentrations. Appearance of neurites induced by ST was similar to that induced by NGF but different in the following property. Down regulation of PKC (assayed by protein phosphorylation, 3H-St binding and fluorescent phorbol ester imaging) by TPA (500nM-2hrs) or inhibition of kinases by K-252a family of compounds, had no effect on neurite outgrowth in PC12 cells. This suggests that the neurotrophic effect of ST (at doses that inhibit PKC) is unrelated to the inhibition of PKC.

Elucidation of these mechanism(s) has clinical implications and is important in exploring NGF action in the nervous system.

- Supported by ICRF and Isr. Nat. Inst. for Psychobiol.


We recently have reported that extracts 2.5x nerve growth factor (NGF) stimulates neurite outgrowth in vitro of identified motor neurons and interneurons of the pond snail, Lymnaea stagnalis (Ridgway et al., J. Neurobiol., in press). Antiserum generated to NGF was found to block the spontaneous outgrowth of cells, possibly by immunoblotting and immunochemistry methods. Culture medium conditioned with Lymnaea central ganglionic rings (2/ml) for 72 h was concentrated 20 fold, via 10K exclusion filters (YM-10) filtration membranes, subjected to gel electrophoresis, and then transferred to nitrocellulose for immunoblot analysis. Prominent polypeptide bands with relative molecular weights (Mw) of 183, 185, 164, 137 and 124kDa (under reducing conditions) were identified using three different antibodies to NGF; some bands may represent proteolytic fragments of a larger protein. Chromatography of conditioned medium on an ion exchange column (DEAE) suggested a NGF-like immunoreactive molecule. We conclude that the concentration of this molecule in CM can be enhanced by glutamate. One of the key questions arising from these observations is the nature of the mechanism by which glutamate enhances CM (e.g., does it increase the synthesis and/or release of NGF?) we show that Lymnaea brain-conditioned medium (CM) contains a NGF-like immunoreactive molecule. Here we demonstrate that the molluscan nervous system is modulated by glutamate. Supported by the Canadian Centre for Excellence in Neuroregeneration and Functional Recovery.
Mn\textsuperscript{2+} INDUCES NEURON-LIKE MORPHOLOGICAL CHANGES IN PC12 CELLS. J.W. Lin*, K. A. Marques* and J.A. Roth, Dept. of Pharmacology and Therapeutics, School of Medicine and Biomedical Sciences, SUNY at Buffalo, Buffalo, NY 14214

Divalent cations in the culture medium have been shown to affect the attachment of PC12 cells to the substrate. However, their effect on the process of neuronal differentiation have not been characterized. When cells were treated with 0.3 - 2.0 mM of MnCl\textsubscript{2}, we observed morphological changes similar to those induced by K\textsuperscript{+} ions, i.e. flattening of cell bodies and extension of neurites. These responses were dose-dependent and observable within 6 hours after treatment. The effects of Mn\textsuperscript{2+} were enhanced in medium containing 1% serum and in the presence of NGF. Of the other divalent cations examined, only Ca\textsuperscript{2+} was able to simulate the effects of Mn\textsuperscript{2+}. When compared to the morphological changes observed with forskolin, the Mn\textsuperscript{2+}-treated cells were appreciably flatter, with cell body diameters up to 30 \mu m. While forskolin alone induced neurite outgrowth maximally in only 50% of the cells, co-incubation with submaximal concentration of MnCl\textsubscript{2} increased the response rate to over 90%, with a profound increase in the size of the cell bodies. In addition, Mn\textsuperscript{2+} was also able to induce similar responses in a mutant PC12 cell line deficient in protein kinase A activity. These observations suggest that Mn\textsuperscript{2+} induces neurite outgrowth in PC12 cells through a mechanism independent of cAMP. Receptors remain as to whether NGF and Mn\textsuperscript{2+} share a common signal transduction pathway which results in morphological differentiation of PC12 cells. (Supported by NIH grants NS 20530 and ES 04249)

ELEVATED POTASSIUM MAINTAINS NEURITE STABILITY IN A PC12 MUTANT CELL LINE AFTER NGF WITHDRAWAL. KENNETH K TENG AND LLOYD A GREENE*. Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, New York 10032

We have isolated a clonal PC12 mutant cell line designated Clone 41 (C41), with heightened responses to NGF. Compared to wild type PC12 cells, the C41 line extends neurites in significantly greater abundance in the presence of NGF. Biochemical analysis demonstrated that differentiated C41 cells differ from their PC12 counterparts in showing more pronounced changes in cytoskeletal proteins. In characterizing the C41 line, we observed that it is not NGF dependent (0.8mM) can maintain neurites for at least 3 weeks after NGF withdrawal; however although they are maintained, the neurites do not continue to elongate. We have also shown that the neurites of C41 cells are most likely a complex containing the 35 kDa protein. NI-250 is a complex containing the 35 kDa protein. NI-250 is a complex containing the 35 kDa protein. NI-35 is a complex containing the 35 kDa protein. These results show that NI-35 and NI-250 are closely related proteins and that the neuronal degeneration. Cells in which NGF is replaced with K\textsuperscript{+} also contain a "primed" state characterized by rapid NGF-dependent neurite reneuritogen. The K\textsuperscript{+}-induced effect is inhibited by Ca\textsuperscript{2+} channel blockers (Verapamil and/or Nitrendipine). This neurite-stabilizing effect is accompanied by an alteration in several cytoskeletal components. Amongst these, phospho-β-tubulin decreases to undifferentiated levels while the microtubule associated proteins MAP1.2 and 1.3 remain highly phosphorylated in NGF-treated controls. The data presented here suggest that depolariization may stabilize neurites by the absence of neurotrophic factors, in part at least by affecting the cytoskeleton.

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Initial N-terminal and internal amino acid sequence data of peak II derived from NI-35 or NI-250 migrated at 33 kDa on SDS-PAGE. The amino acid compositions of peak I and II were determined and found to be very similar. These results show that NI-35 and NI-250 were identical to the corresponding sequences of peak II of NI-250. The size of the cell bodies. In addition, Mn\textsuperscript{2+} was also able to induce similar responses in a mutant PC12 cell line deficient in protein kinase A activity. These observations suggest that Mn\textsuperscript{2+} induces neurite outgrowth in PC12 cells through a mechanism independent of cAMP. Question remains as to whether NGF and Mn\textsuperscript{2+} share a common signal transduction pathway which results in morphological differentiation of PC12 cells. (Supported by NIH grants NS 20530 and ES 04249)

598.9

INDUCTION OF GROWTH CONE COLAPSSING ACTIVITY IN CULTURED ASTROCYTES BY FGF AND INTERLEUKIN 1. Alan R. Hughes, Department of Developmental Neurobiology, Stanford University School of Medicine, Stanford, CA 94305

Regeneration of the peripheral nervous system requires the precise reformation of synaptic connections between motor and sensory neurons and the tissues that they innervate. Schwann cells are essential to this process. To explore the Schwann cell-neuron interaction, we have established primary cultures of adult rat Schwann cells from crushed sciatic nerves. From these primary cultures, we have isolated a spontaneously immortal Schwann cell clone (ISC), whose conditioned media (CM) contains a neurite-pro-moting activity that is not active on PC12 cells. Embryonic chick ganglia explants were incubated with CM to determine the neuronal specificity of this activity. This activity was found to be less selective for a general class of neurons, neurites are elicited from CNS (spinal cord, brain and retina) and PNS (dorsal roots and sympathetic chains ganglia). We have characterized this activity after elution from a cation exchange column. In a dilution of 1:50, this eluate retains its neurite-promoting activity with embryonic neurones. Neither the column profile nor the types of embryonic neurones supported by this eluate are consistent with it being CMTP. This activity does not bind to heparin and antibodies to bFGF do not inhibit neurite promotion. Although poly-Adenosine RNA from ISC gives a positive signal when probed with cDNA for NGF, blocking antisera to NGF does not inhibit neurite formation. This Schwann cell clone has been transplanted to a time of trophic activity and may provide new information on the regenerative milieu.

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NEURITE PROMOTING ACTIVITY FROM AN IMMORTAL SCHWANN CELL CLONE. M.M. Holin and E.M. Shooter. Dept. of Neuroscience, The Weizmann Institute of Science, Rehovot, Israel

A NEURITE PROMOTING ACTIVITY FROM AN IMMORTAL SCHWANN CELL CLONE. M.M. Holin and E.M. Shooter. Dept. of Neuroscience, The Weizmann Institute of Science, Rehovot, Israel

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PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF RAT AND BOVINE CNS MYELIN ASSOCIATED NEURITE GROWTH INHIBITORS NI-35 AND NI-250. C.E. Bandtlow and M.F. Schwab. Brain Research Institute, University of Zurich, 8092 Zurich, Switzerland

Rat and bovine CNS myelin contain two proteins of 33-35 kDa and 250 kDa which exert potent inhibitory effects on extending neurites (neurite growth inhibitors N1-35 and N1-250). SDS-PAGE eluted N1-35 and N1-250 revealed several active spots ranging from 44- to 62-kDa proteins. The 35- and 250-kDa proteins in hydrophobic proteins; so we further purified this material on a C4 reverse phase HPLC column. The chromatograms, showing major protein peaks (1 and II) were identical for both, N1-35 and N1-250. Peaks I and II, derived from N1-35 or N1-250 migrated at 33 kDa on SDS-PAGE. The amino acid compositions of peaks I and II were determined and found to be very similar. Initial N-terminal and internal amino acid sequences of peaks I and II were identical to the corresponding sequences of peaks I and II. The amino acid sequences available so far show no homologues to known proteins.

These results show that N1-35 and N1-250 are closely related proteins and that N1-250 is most likely a complex containing the 35 kDa protein. N1-35 is a microheterogeneous consisting of 2 subfractions differing slightly in their hydrophobicity.


599.3

COMPARISON OF RABBIT FACIAL NERVE REGENERATION IN NGF-CONTAINING TUBES TO AUTOGRAFTS. S. R. Leach, L. Spector, G. Danger, and H. Deckert. Department of Psychology, Washington University, St. Louis, MO 63130.

Previous reports suggest that NGF enhances rabbit facial regeneration. We compared facial nerve regeneration in NGF or Cytochrome C (Cyt. C)-containing allisitic tubes to autologous nerve grafts. A 4.5 mm segment of the buccal division of the facial nerve was removed, the nerve ends were inserted into a 10 mm tube and sutured to the corresponding defect on the opposite side. Five weeks post-injury, the peripheral nerve terminals were assessed by morphological evaluation. NGF treated nerves recovered significantly higher percentage of their preoperative number of myelinated fibers than Cyt. C treated nerves (46% vs 18%). The number of myelinated fibers reaching the distal nerve stump was proportionately greater in NGF treated nerves than in nerve grafts. In nerve grafts, the percentage of intraocular myelinated fibers was inversely related to the amount of myelin debris and the majority of fibers (66%) were extrascalar. Thus, although nerve grafts contain larger number of myelinated fibers the NGF-containing tubes, functional recovery and elimination of aberrant reinnervation may not be superior to entubation repair with NGF.

599.4

INHIBITION OF NGF-STIMULATED NEURITE OUTGROWTH IN AMINE-MODIFIED α-MACR OGL OBULIN. D.J. Liebl and P.H. Koo. Neurosciences Program and Dept. of Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44276.

Alpha-2-macroglobulin (a2M) is a rather ubiquitous protein in extraacellular spaces of mammals. It is an inhibitor of endopeptidases and a carrier for a number of cytokines/hormones such as B-nerve growth factor (NGF) (P.H. Koo and R.W. Stach, J. Neurosci. Res. 22:247, 1989). The objective of this study is to determine whether a2M and its modified forms may affect the biological activity of NGF. Alpha-2-M was purified from human plasma and then modified by either methylamine or tryptamine. The results clearly show that methylamine-modified α(Ma αM) exerted a dose-dependent inhibition on the NGF-stimulated neurite outgrowth by embryonic chick dorsal root ganglia whereas normal αM and tryptamine-modified αM had little or no effect. Ma αM is not toxic to neurones and the inhibition can be overcome by higher NGF concentrations. As determined by gel filtration, native polycarylamide gel electrophoresis and SDS-urea gel electrophoresis, both normal αM and Ma αM were >90% of NGF nonspecifically. Although Ma αM carries about twice as much NGF, much higher doses of normal αM had little or no effect on the biological activity of NGF. This suggests that amines modified αM may inhibit neurite growth via an active process not involving passive absorption of NGF.
NERVE GROWTH FACTOR VII 1497

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After the primers were used to PCR amplify normal and schizophrenic human genomic DNA, the resultant double-stranded DNA was restriction-digested and ligated into Bluescript. The circularized vector with NGF insert was transfected into competent E. coli. Resulting white colonies were screened for NGF insert by amplifications of DNA with universal and reverse primers. The positive NGF PCR products were purified using Centricon 100 filters and used for DNA sequencing. Sequence analysis was carried out with fluorescent-labeled universal and reverse primers, using the Applied Biosystems Inc. cycle sequencing linear amplification protocol. Sequencing reactions were analyzed on an ABI 373 DNA Sequencer. Automated NGF gene sequencing has the potential to identify changes in the primary sequence of NGF that may be associated with specific disease states. This work was supported by the VA Medical Research Service and USPHS Grant No. 50 MH4212-02.

NERVE GROWTH FACTOR PREVENTS TOXIC NEUROPATHY. M. E. Maron et al., Albert Einstein College of Medicine, Bronx, N.Y. 10461

Nerve growth factor regulates the survival and function of subpopulations of dorsal root ganglia sensory neurons in vitro and in vivo. We have previously demonstrated the ability of NGF to prevent the small fiber sensory neuropathy caused by administration of the experimental anti tumor agent taxol. In this study we sought to determine whether NGF is also capable of preventing the large fiber sensory neuropathy caused by the widely used anti tumor agent cisplatin. Cisplatin was administered intraperitoneally to adult mice at a dose of 10 mg/kg once a week for a period of six weeks. The animals were sacrificed subcutaneously with either NGF (5 µg/kg) or control buffer three times a week. After the eight-week administration period peripheral nerve function was examined using carefully selected behavioral, biochemical, and electrophysiological techniques. Cisplatin administration impaired proprioception as measured by the ability to balance on a rotating dowel (p<0.005), reduced sensory ganglion levels of the peptide transmitter calcitonin gene related peptide (p<0.02) and slowed nerve conduction in the tail (p<0.006). NGF coadministration prevented all of these abnormalities, indicating that the factor was effective in the setting cisplatin induced neuropathy. We are currently testing the ability of NGF to prevent other diseases of the peripheral nervous system. These observations suggest that clinical trials of the growth factor are now warranted.

NERVE GROWTH FACTOR (NGF) CHANGES IN RAT EXPERIMENTAL DIABETIC NEUROPATHY. R. Hellweg1, H.D. Hartung2, C. Höck1, M. Wörth1, and G. Ravich

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NGF-responsive peripheral neurons with small and unmyelinated fibers are affected at an early stage of diabetes mellitus. As revealed by NGF enzyme-linked immunosorbent assay (ELISA) induced-destruction of insulin-producing pancreatic islets, which causes diabetes mellitus to lead to a 3-fold increase of NGF levels in most NGF-producing tissues (iris, heart atrium and ventricle, spleen, vas deferens), but to a 50% decrease in the NGF-dependent sympathetic superior cervical ganglion1. This decrease in the superior cervical ganglion was reversible by successful pancreatic islet allotransplantation. Since NGF is known to exert its effects after retrograde axonal transport together with NGF receptor, we have studied this axonal transport in the rat sciatic nerve 8 weeks after the induction of diabetes with STZ. The overall retrograde transport of NGF decreased to 50% of untreated controls (p<0.001 in a 2-sided Student’s t-test), and the amount of retrogradely transported NGF in the STZ-treated animals was negatively correlated with the individual blood glucose concentration (r=0.68, p<0.002). Taken together, our results could suggest a pathophysiological role for NGF in diabetic neuropathy which may be due to a decreased neuronal capacity of NGF uptake and retrograde transport. Thus, prevention of diabetes-related NGF deprivation in NGF-dependent peripheral neurons could play an important role in the future therapy of diabetic neuropathy.

NERVE GROWTH FACTOR RECEPTOR INDEX IN ALZHEIMER’S DISEASE. J. M. Khachaturian, R. Weinberger

1University of California, San Francisco, CA 94117, 2Stanford University School of Medicine, Stanford CA 94305

The expression of the nerve growth factor (NGF) receptor is increased in Alzheimer’s disease. To determine the extent of receptor expression, we examined the frontal, temporal, and parietal cortices of 27 cases of Alzheimer’s disease (including 18 normal controls) using a monoclonal antibody raised against a 180 kDa protein. Staining patterns were compared with those obtained on sections from the same brain using the S IS (situation) antibody. This antibody recognizes a 70 kDa protein in normal brain and a 100 kDa protein in Alzheimer’s disease. We found that the expression of the NGF receptor was increased in all three regions, with the greatest increase observed in the parietal cortex. The S IS antibody labeled the same regions, but with the same intensity as in normal brain. In addition, there was a significant correlation between the levels of NGF receptor and the severity of the disease as determined by the Clinical Dementia Rating Scale (r=0.62, p<0.01). These results suggest that the expression of the NGF receptor may be related to the pathogenesis of Alzheimer’s disease.

CHROMATOLYTIC-LIKE ALTERATIONS AND ABERRANT NEUROFILAMENT PHOSPHOPROTEIN INSENSITIVITY IN SENSORY NEURAL PERIKARYA FOLLOWING NGF ANTISERUM INJECTION. B.G. Gold, D.R. Austin*, W.C. Mobley*, and J. Storm-Dickerson*


The mechanism by which the neuronal perikaryal response to injury (axon reaction) is initiated is unknown. We are utilizing nerve growth factor (NGF) antisera injections for 2 weeks (Gold et al., J. Neurosci. 11:943, 1991) demonstrated that loss of NGF triggers two components of the axon reaction (i.e., somatofugal axonal atrophy and nuclear eccentricity). In the present study 4-week-old male rats were given daily injections of goat-antimouse NGF antisera (0.5 µg/ml) into the left footpaw for 4 weeks; age-matched control animals were injected with control goat serum. The resulting endoplasmic reticulum was displaced to the periphery of the cell body in some (5-10%) L4-L6 DRG neuronal perikarya. Eccentric nuclei demonstrated prominent nuclear membrane indentations. Some neurons showed accumulations of lysosomes and lipofuscin granules. Axonal atrophy, present only at the level of the DRG at 2 weeks, was observed in the distal portion of the dorsal roots, demonstrating its somatotopic progression. Preliminary immunocytochemical studies (peroxidase-antiperoxidase method) revealed light to modest immunoreactivity in neuronal perikarya (less than 30%). Though the pathogenic action of NGF is essential for the development and maintenance of neural crest derived sensory, sympathetic and basal forebrain cholinergic neurons, recently it has been shown that alpha motor neurons may also utilize NGF. These neurons express nerve growth factor receptors (NGFR) developmentally and in response to mechanical injury to peripheral axons. Because of these findings, we were interested in the role of NGF in the alpha motor neuron response to pathological degenerative processes. We also demonstrated that alpha motor neurons may express heat shock protein (HSP) mRNA, which may identify vulnerable cells. An in vitro hybridization procedure was used to localize alpha motor neurons expressing NGF mRNA and HSP mRNA in posterior spinal cord from patients with amyotrophic lateral sclerosis (ALS). Cervical, thoracic, and lumbar sections were processed with a radioactive 2.5 kb cDNA probe complementary to rat NGF mRNA, or a 30-mer oligonucleotide probe complimentary to HSP mRNA. Additional sections were processed using cresyl violet or silver degeneration staining procedures. The expression of NGF mRNA in the ventral horn was greatest at those spinal cord levels which exhibited the most extensive cell loss and atrophy, and which were associated with the most extreme clinical symptomatology. In addition, HSP mRNA was localized primarily to a few ventral horn cells in all segments analyzed. We hypothesize that as the disease process advances, some motor neurons start to re-express growth related proteins in an attempt to compensate for the progressive degenerative state. This is supported by EMG and histochecmical evidence demonstrating the reinervation of denervated muscle via collateral sprouting in ALS patients. Supported by PHS grant AG-08999 (GIS).

DETECTION ANALYSIS OF MOUSE NERVE GROWTH FACTOR. C. C. Dinkinswater, U. Soter, & R. D. Shoppert

Dept. of Neurobiology, Stanford University School of Medicine, Stanford CA 94305

Nerve growth factor (NGF) is a trophic peptide responsible for the survival, development, maintenance, and regeneration of neural-crest derived sensory and sympathetic neurons as well as basal forebrain cholinergic neurons. Binding sites and mRNA for NGF have been detected throughout the developing avian and mammalian central nervous system, indicating that NGF may affect other cell types early in development. The primary structure of Ngf from several different species has been determined by amino acid and nucleotide sequencing; however, only one three-dimensional structure has been completed. NGF is initially synthesized as a 33.8 kDa precursor, from which the mature form is cleaved by one or more trypsin-like enzymes. In mouse, active NGF appears to comprise a non-covalently bound homodimer of 118 residue chains, each with three internal disulfide bridges, to define a minimal structural unit of NGF with biological activity. A series of deletion mutants were constructed, using DNA in vitro mutagenesis. These mutants were expressed in Escherichia coli and analysed for biological activity. The yields of two mutants were significantly reduced in comparison to wild type recombinant NGF, and no activity could be detected in a neurite outgrowth assay. Though the third mutant, in which amino acids 3-14 were deleted, was produced in relatively high amounts, it was not correctly processed to the mature, active form. Treatment with trypsin does not appear to activate this mutant precursor. Therefore, this region may be critically involved in the specific processing of NGF from its precursor. We are currently examining this region in greater detail by further in vitro mutagenesis.
A RAT CELL LINE PRODUCING RECOMBINANT HUMAN NERVE GROWTH FACTOR (rhNGF) has been obtained by transfection of El5 Sprague-Dawley rat fibroblasts with the human NGF gene using MRE(4X)-OVEC vector. These cells were tested for NGF production (approximately 0.5-1.0 ng/ml of conditioned medium) by a biological assay using fibroblast outgrowth from embryonic sympathetic ganglia, as well as a two-site enzyme immunoassay for NGF. RNA was isolated from these cells using phenol extraction, and the corresponding NGF mRNA was detected by Northern blot analysis. These studies indicate that the rhNGF gene is stably integrated into the rat genome and produces biologically active NGF.

A functional domain of the low affinity NGF receptor (LNGFR) has been identified in the putative extracellular domain of the receptor. Using a series of truncated constructs, it was shown that the LNGFR consists of the NGF-inducible nerve growth factor receptor (NGFR) and a second component (LNGFR) that modulates the biological effect of NGF on responsive cells. The LNGFR may modulate the biological effect of NGF on responsive cells by controlling the downstream signaling pathways activated by NGF.

The hippocampus is the major target region for glucocorticoid action in the nervous system. Adrenalectomy results in a nearly complete cell loss of hippocampal granule cells 3 to 4 months after surgery. In the present study, we investigated the effect of glucocorticoids on neurotrophic factor mRNA expression in the hippocampus. The mRNA levels of NGF, BDNF, and NT-3 were measured by quantitative RT-PCR. The mRNA levels of NGF, BDNF, and NT-3 were significantly decreased in the hippocampus of adrenalectomized rats compared to intact animals. These data suggest that glucocorticoids are involved in the regulation of neurotrophic factor mRNA expression in the hippocampus.
600.1 MORE REGENERATED AXONS IN THE DISTAL STUMP OF TRANSECTED SCOTIC CORD WITH GROWTH FACTOR. B. V. K. Harman*, J. K. B. Korner*, R. Lim, J. G. de la Torre, Univ. of Ottawa, Ontario Canada K1H 8M5

Sixteen female Sprague-Dawley rats were anesthetized and prepared for bilateral sciotic nerve transection. The transection and embolization treatment involved: pre-treating the nerve with 15% poly vinyl alcohol/chloroprene solution, to remove myelin and protein coating of axon tips; rapid cooling with fluoromethane spray, to render the nerve firm; transection with an ultrafine-edge razor blade. In each animal, the 2 mm (± 0.25 mm) stump gap created was filled with either collagen only (COL) or a collagen/neurotrophic (CONIF) factor mixture. Each NTf had the prospect of exerting a stimulatory or protective effect on regenerating axons when applied at the transection and repair site and were chosen because they were not yet tested in the sciatic nerve. The concentration of each NTf/ini collagen was: 4-aminopyridine (4-AP) 0.2 mg; lipid argiogenic factor (LAF) 2.0 mg; leupeptin (LEU) 0.2 mg; 8-GMF 20 mg. After 6 weeks the number of regenerated myelinated axons in the distal stump was counted using our line-sampling quantification technique, and compared to the controls (COL). We found that nerves treated with 4-AP, LAF and LEU had both greater and fewer axons. However, in the distal stumps of the nerves treated with 8-GMF, there were consistently more axons (48-52%). We therefore conclude that 8-GMF may stimulate regeneration in the peripheral nervous system. The implantation of a COL/NTF mixture in our embolization preparation provides a useful and quantifiable technique for screening potential enhancers of peripheral axon growth.

Funded by the Easter Seal Research Institute (Ont.).


Reactive differentiation of astrocytes is a key component of the central nervous system response to injury. As such, the factors that cause the metabolic changes associated with cellular reorientation, 2-deoxyglucose (2DG) uptake was measured in cultures treated with agents known to induce morphologic reconfiguration or impede proliferation. Secondary rat astrocyte cultures were treated with dibutyryl cyclic AMP (dBcAMP), a cerebral tissue extract with morphogenic properties or ethanol levels sufficient to diminish proliferation. The 2DG uptake was measured using the method of Brooks and Yarovsky (1985, J. Neurochem. 44:473-479).

Increased 2DG uptake was detected in control cultures at 3 and 6 hrs after replenishing the nutrient medium, but uptake values returned to baseline at 24 hr. Treatment with either dBcAMP (1mM) or cerebral extract (1mg/ml) enhanced 2DG uptake at 3, 6 and 24 hrs. These changes in 2DG were noted prior to qualitative changes in astrocytic morphology. The increase in 2DG uptake induced by treatment with dBcAMP and cerebral extract (0.5 mg/ml) was sustained at 24 and 48 hrs of treatment. Simultaneous exposure to 0.2% or 0.5% (w/v) ethanol augmented this response to dBcAMP and cerebral extract at 24 hr. These data suggest that metabolic changes requiring glucose consumption precede alterations in astrocytic morphology and may have a role in reactive differentiation.

Supported by NIAAA grant AA07145.

600.3 NEURONAL SURVIVAL OF CEREBELLAR GRANULE CELLS IN A COMPARTMENTED CULTURE WITH DIGESTED INTRACELLULAR CALCIUM. T.Koike, Department of Natural Science, Saga Medical School, Nishiharu, Saga 84001, JAPAN.

We have previously proposed a calcium set-point hypothesis for the degree of neuronal dependence on trophic factor (in vivo) (T.Koike et al. PNAS, 86:6421, 1989), and provided evidence for this hypothesis in sympathetic neurons (T.Koike and S.Tanaka, ibid, 88, in press, 1991). Here, as an extension of this study, we examined the relationship between intracellular calcium ([Ca2+]i) and survival of cerebellar granule neurons. To this end, we created a compartmented culture system of mixed cultures, consisting of neurons and glial cells, in which neuronal death due to unavailable trophic factor that occurs in the absence of trophic factor (in vivo) can be mimicked in vitro. Here, as an extension of this study, we examined the relationship between intracellular calcium ([Ca2+]i) and survival of cerebellar granule neurons. The granule neurons isolated from newborn rat cerebellum were cocultured with neuronal growth in serum-free medium and used for the assay. The neuronal death due to trophic factor deprivation that occurs in vivo can be mimicked in vitro.

Using this system, we have found that the neuronal death due to trophic factor deprivation that occurs in vivo can be mimicked in vitro. The neuronal death due to trophic factor deprivation that occurs in vivo can be mimicked in vitro.

Supported by NIAAA grant AA07145.

600.4 NICOTINIC AGONISTS, PHORBOL ESTERS AND GROWTH FACTORS ACTIVATE DISTINCT MYELIN BASIC PROTEIN KINASES IN BOVINE CHROMAFFIN CELLS. B. Pavlović-Surijavec*, A. L. Cahill, R. L. Perlman, Deps. of Pediatrics and Pharmacol. and Physiol. Sciences, The University of Chicago, Chicago, IL 60637.

Insulin-like growth factor I (IGF-I) increases protein kinase activity against microtubule-associated protein-2 and myelin basic protein (MBP) in bovine chromaffin cells (Chapman et al., PNAS, 86:6421, 1989, in press). To characterize the MBP kinases in chromaffin cells, we separated chromaffin cell proteins by electrophoresis on SDS-polyacrylamide gels into which MBP had been incorporated, allowed the proteins to renature and then assayed MBP kinase activity by incubating the gels in solution containing [32P]-ATP. We have identified six MBP kinases, with Mr of 116, 80, 53, 46, and 42,000. The MBP kinases of Mr 46,000 and 42,000 (PK46 and PK42) were activated by treatment of the cells with the nicotinic agonist dihydro-β-erythroidine (DHβE) or IGF-I. The involvement of protein kinase C (PKC) in the activation of PK46 and PK42 was examined. Down regulation of PKC by incubation of the cells with PD98 abolished the activation of both PK46 and PK42 by DMPP, PD98 and IGF-I. Stauroporine, a PKC inhibitor, prevented the activation of PK46 and PK42 by DMPP and PD98, but did not block the activation of these kinases by IGF-I. Thus, chromaffin cells contain two MBP kinases that can be activated by both PKC-dependent and PKC-independent mechanisms. These kinases may participate in protein kinase cascades activated by various agonists. (Supported by research grants from NIH and NSF).

600.5 CHOLINERGIC DIFFERENTIATION OF CULTURED RAT SYMPATHETIC NEURONS BY FACTORS APPLIED TO DISTAL NEURITE. D. B. Ure, R. R. Campenot, and A. Abelson, Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

The development of cholinergic properties in rat sympathetic neurons in vivo is believed to occur in response to factors derived from target tissues. Although the cholinergic switching event in mass cultures occurs in response to a variety of factors, only one model, the rat mesentery ganglion, directly tests the theory of target-mediated cholinergic differentiation. The results of our studies using a variety of factors applied to rat sympathetic neurons in a compartmented culture system (in vivo) also support that theory. When 600 units/ml leukaemia inhibitory factor (LIF; Daclita cell line proliferation assay, 30,000) or 50% dermal fibroblast conditioned medium are supplied to only distal neurites from Day 7-21 in culture, a many-fold increase in a specific choline acetyltransferase (ChAT) activity and a decrease in tyrosine hydroxylase immunoreactivity are observed. The increase in ChAT activity in time- and concentration-dependent. Our results suggest that LIF and the factor(s) in conditioned media could act as target-derived differentiation factors in vivo.

600.6 NOT ALL CNS TRANSPLANTS CAN RESCUE AXOTOMIZED CLARKE'S NUCLEUS NEURONS. B.T. Himes*, P. Baker, M. L. Goldberger1, and A. Teitel2 Dept. of Anatomy and Neurobiology, The Medical College of Pennsylvania1, and VA Medical Center2, Philadelphia, PA.

Axotomy of Clarke's nucleus (CN) results in a 50% loss of CN neurons in neonatal rats and a 30% loss in adults 2 months postoperatively. This retrograde degeneration is a reaction to the loss of target derived neurotrophic factors, which have not been identified in CN. CN axotomy, when compared to the retrograde degeneration in a compartmented culture system, also support that theory. When 600 units/ml leukaemia inhibitory factor (LIF; Daclita cell line proliferation assay, 30,000) or 50% dermal fibroblast conditioned medium are supplied to only distal neurites from Day 7-21 in culture, a many-fold increase in a specific choline acetyltransferase (ChAT) activity and a decrease in tyrosine hydroxylase immunoreactivity are observed. The increase in ChAT activity in time- and concentration-dependent. Our results suggest that LIF and the factor(s) in conditioned media could act as target-derived differentiation factors in vivo.

Laminin, a large glycoprotein consisting of three polypeptide chains, is an extracellular protein that has been implicated in promoting Schwann cell neurite outgrowth. Laminins are known to act as potent stimulators of neurite extension and can enhance neuronal survival in vitro. In vivo, its presence on the surface of Schwann cells has been correlated with the advancement of neurites regenerating across a 10 mm gap (Longo et al., Brain Res. 309:105-107). We examined regenerating rodent peripheral nerves and their associated sensory neurons for changes in laminin mRNA expression in situ by hybridization with sense or anti-sense laminin B2 RNA probes. Regenerated sciatic nerves showed an up-regulation of laminin B2 mRNA in Schwann cells compared to control sciatic nerve. More surprisingly, we detected laminin B2 mRNA in L4 and L5 DRG neurons and this mRNA appeared to be up-regulated during regeneration. Increased laminin B2 mRNA expression was also observed in the satellite cells of those DRGs after sciatic nerve injury. These findings suggest that neurons, as well as supporting cells, may be capable of regulating the synthesis and secretion of a glycoprotein implicated in their own as well as neighboring cells differentiation, migration, and in the case of neurons, neurite elongation. Immunohistochemical studies are currently in progress to determine the relationship between the synthesis and deposition of laminin during peripheral nerve regeneration.


In an ongoing study of agents with potentially trophic effects upon spinal cord neurons, we have safety-tested intrathecal uridine (U) and orotic acid (OA), two precursors of RNA via the salvage de novo pathways, respectively. In U/kg/d ranging from 410 to 1640, and OA, from 440 to 3520, were administered to young male and female rabbits with ailet osmotic pumps, through a catheter inserted into the spinal subarachnoid space at the L7-S1 interasse. Freshly loaded pumps were reimplanted at two-week intervals for as long as tolerated.

Of the two, OA seemed better tolerated: 3520 U/kg/d for over five mos., and one rabbit tolerated 880 U/kg/d for over 9 mos., without discernable side effects. U was well tolerated, for over 20 mos., at 820 U/kg/d, but with 1640 U/kg/d, diarrhea and weight loss occurred after 9 wks. The animals continued to maintain normal growth and behavior throughout the treatment periods, but at a reduced rate with the higher dosages of OA & U, respectively 3520 and 1640 U/kg/d.

Thus, OA & U will probably be safe, within the dose ranges noted above, for intrathecal administration as potential therapeutic agents for human neurological disorders.


During the early stages of auditory development, the otocyst releases a diffusible trophic factor which promotes the outgrowth of neurites from statoacoustic gangli in (SAG). The present experiments evaluated the developmental time course of this trophic influence by coculturing E4-E15 SAG explants with otocysts of various developmental stages. In the absence of otocysts, SAG was seen from SAG explant at all stages. However, extensive neurite outgrowth occurred when E4-E6 SAG were cultured in the presence of otocysts of the same age. The ability of SAG to promote neurite outgrowth from otocysts was maintained throughout development. Neurite outgrowth from E10-E15 SAG was promoted in the presence of the conditioned factor released by younger stage (E4) otocysts. The results of our experiments indicate that there is a developmentally-regulated release of a diffusible chemotrophic factor from the otocyst which influences neurite outgrowth from SAG. (Supported by grants from the Deafness Research Foundation and NIH #NS25785)


We studied dissociated cell cultures from the locus coeruleus, substantia nigra and cerebral cortex of postnatal rats to determine if Schwann cells (SCs) and SC products promote survival and regeneration of specific types of postnatal CNS neurons (NCs). We used an image analysis system to measure several morphometric parameters of NCs to assay for the effects of SCs or SC conditioned medium (SCM) on the regenerative capacity of surviving NCs. NCs were identified by immunostaining for Tau, TH, and antitau and anti-TH antiserum (TH). Two populations of NCs were identified by their immunoreactivity for Tau and/or NF: Tau positive (Tau+) alone or both NF and Tau positive (NF/Tau+). In addition, a third population of NCs which was both TH and NF immunoreactive (TH/NF+) was identified in cultures from brainstem nuclei. Neuronal survival of all three populations was increased at least 1.5 fold in the presence of living SCs and in SCM as compared to appropriate controls, including neurons grown in the presence of fibroblasts. Interestingly the greatest effect of SCs and SCM was seen on the NF/Tau+ subpopulation. SCs or SCM increased the number of these NCs by at least 10 fold. Image analysis of NF/Tau+ NCs revealed an increase in somal perimeter, mean number of primary neurites, mean number of processes, total neurite length, and the extent of neuritic field in the presence of SCs or SCM. Filtration and dialysis experiments appear to indicate that the active components in SCM are ≥ 10kDa. Our results suggest that SCs and soluble SC products support both the survival and regenerative capacities of several specific classes of postnatal CNS neurons. (Supported by NS24252).


Recent data have suggested that $100G$ is a bifunctional growth/differentiation factor, stimulating proliferation of glial cell and survival and morphological differentiation of immature neurons. We demonstrate here that dimeric $100G$ can evoke increases in free, intracellular calcium concentrations ($Ca^{2+}$) in both glial and neuronal cells. The $100G$-induced $Ca^{2+}$ increase included a rapid, transient component that was independent of extracellular calcium and a sustained, reversible elevation that was dependent on calcium influx. The monomeric form of $100G$ appeared to lack the ability to evoke this response. The $100G$-induced $Ca^{2+}$ increase was blocked by an inhibitor of phospholipase C, consistent with the ability of $100G$ to stimulate the hydrolysis of phosphatidylinositol. In at least one neuronal cell type, $100G$ stimulated increases in $Ca^{2+}$, synergistically with high K*. These data demonstrate that while the biological responses of neuronal and glial cells to $100G$ are quite different, transduction of the $100G$ signal in both cell types may involve changes in $Ca^{2+}$, (Supported by Muscular Dystrophy Association).
A protein purified and cloned in our laboratory based on its co-purification with the 52kD Acetylcholine- Receptor-Inducing Activity shares a 33% homology to the mammalian prion protein (Halls et al. CSH Syn, 55, 1990). We report here the distribution of this chick prion-like-protein (ch-PLP) in the chicken spinal cord. Sequence comparison of a partially sequenced insert localized c-fos messenger RNA (mRNA) in postmortem monkey (M. fascicularis) cerebral cortex, hippocampal subfields, and the Purkinje cell layer of cerebellum. Many of these regions involve with neurodegenerative disorders, we sought to represent a third messenger system and participate in key cellular regulatory pathways. Since proto-oncogenes following membrane depolarization and calcium influx, and exposure to nerve growth factor and cholinergic agonists. Since proto-oncogenes play a role in cell growth and differentiation, the expression of c-fos is regulated by a number of genes (e.g. beta-amyloid precursor protein, tyrosine hydroxylase, SV40). In addition, the expression of the c-fos is regulated by a number of physiological parameters: induction of c-fos occurs following membrane depolarization and calcium influx, and exposure to nerve growth factor and cholinergic agonists. Since proto-oncogenes represent a third messenger system and participate in key cellular regulatory pathways, alteration of which genes occur in the brain regions involved with neurodegenerative disorders, we sought to determine whether c-fos mRNA might be examined in post-mortem human brain. We have used semi-quantitative in situ hybridization (ISH) to localize c-fos messenger RNA (mRNA) in postmortem mouse (M. musculus) and normal human cerebral cortex. In order to compare c-fos expression with Fox protein, we utilized immunocytochemical techniques. The data demonstrate detectable c-fos mRNA and Fox protein in dentate gyrus of hippocampus, as well as hippocampal subfields, and the Purkinje cell layer of cerebellum. Thus, the role of proto-oncogenes in the pathophysiology of human neurodegenerative disorders may now be characterized utilizing postmortem human brain. (Supported by NIH grants AG03644 and NS02032).

It is a well established fact that the calbindin-D-28k gene can be induced by vitamin D in intestine and kidney. However, attempts to modulate this gene in the brain using vitamin D and its metabolites, even at pharmacological doses, have been unsuccessful. While screening for retinoid-sensitive genes in immortalized astrocytes we have recently transfected with an expression vector (pSG5RAR) harboring a human retinoic acid receptor (RAR), we found that retinoic acid (2x10^{-7}M) for 3 days induced the relative abundance of an mRNA which cross-hybridized with human brain calbindin-D-28k cDNA. A 5-8 fold enhancement was observed using both blot and Northern blot analyses and this enhancement was detectable only in cells over-expressing RAR mRNA. Cross hybridization was observed in non-transfected cells. However, the signal was weak and no change was observed after treatment with retinoic acid. Western blot analyses using an antibody specific for rat calbindin-D28k indicated the stimulated presence of an immunologically similar protein with an apparent molecular weight of 29,000 in retinoic acid-treated, retinoic acid receptor transfected cells. Collectively, these data suggest that the nuclear retinoic acid receptor is able to target "calbindin-like" genes in cells derived from the neural crest.


Cholinergic differentiation of cultured rat sympathetic neurons is promoted by several factors including ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and a membrane associated factor (MANS). However, their post-receptor signalling mechanisms are unclear. Here, phorbol 12, 13-myristate acetate (TPA) was used to implicate calcium and protein kinase (PKC) in neurotrophic factor action. Short-term TPA treatment (0.5 μM, 15 min) reduced cytosolic PKC levels by 70% with a corresponding 91% increase in membrane enzyme of PKC was followed by down-regulation of total cellular enzyme levels in chronic TPA-treated (30nM, 1-2 wks) cultures to 14% of control levels. CNTF (0.5 ng/ml) and LIF (0.5 ng/ml) both significantly increased choline acetyltransferase (ChAT) activity by 4.5 and 1.7 fold, respectively. Additionally, neurons grown at double cell density exhibited a 19 fold increase in ChAT activity. In cultures depleted of PKC activity by chronic TPA treatment, co-treatment with LIF (0.5 ng/ml) and CNTF (0.5 ng/ml) or increasing cell density failed to increase ChAT levels. An increase in oral 4a-phorbol, did not affect PKC levels or trophic factor-mediated increases in ChAT activity. Phosphatidylinositol 4, 5-bis-phosphate (PIP2) hydrolysis and the generation of inositol triphosphate (IP3) and diacylglycerol (DAG) is thought to be an initial step in PKC activation. However, LIF or CNTF did not stimulate PIP, hydrolysis as compared to carbachol which increased PIP2 hydrolysis by 268%. These results implicate PKC in neurotrophic factor signalling processes and cholinergic development in sympathetic neurons but activation of PKC is likely to involve alternative sources of DAG such as phosphatidylinositol.

PRIMATE SCHWANN CELLS EXPRESS MULTIPLE GROWTH FACTORS IN VITRO. M.E.D. Nöster, D.L. Hudley, D.M. Gath, and J.T. Hansen. Department of Neuroanatomy and Anatomy, University of Rochester Medical Center, Rochester, NY 14642 and Department of Anatomy, Tulane University Medical Center, New Orleans, LA 70112.

Aged primate Schwann cells have been shown by us to respond to specific mitogens in vitro and express appropriate cell markers such as nerve growth factor receptors, NGF, and IGF-1. Cultures of normal and genetically altered primate Schwann cells were harvested from sciatic nerve, cultured, and partially purified. Both sets were treated at 3 weeks with 2-8.10^{-6}M TCP. After 10 weeks, progressive neuronal necrosis was observed in control whereas TCP-treated cultures survived up to 6 months. In all models, neurons were immunoreactive for GAP-43 and N. S. enolase. However, the TCP-treated neurons showed at the ultrastructural level a marked increase in the length of the processes, in the number and size of growth cones and in the degree of maturation of synaptic. To conclude, TCP appears as a promising drug for the protection and long term survival of neurons.


Experimental evidence supporting for the excitotoxic theory involving the efferent axons of motoneuron and cell bodies in cell culture models. NMDA antagonists are considered as potential neuroprotective agents in neurodegenerative disorders related to excitatory mechanisms. The results reported here show that TCP, (N-[(2-thienyl)cyclohexyl] piperidine, a non-competitive NMDA antagonist enhances the survival of human fetal spinal cord cells in cultures. The spinal cords from 7-10 week-old human fetuses were cultured as either dissociated cells or organotypic explants. Both sets were treated at 3 weeks with 2-8.10^{-6}M TCP. After 10 weeks, progressive neuronal necrosis was observed in control whereas TCP-treated cultures survived up to 6 months. In all models, neurons were immunoreactive for GABA, CAT and N. S. enolase. However, the TCP-treated neurons showed at the ultrastructural level a marked increase in the length of the processes, in the number and size of growth cones and in the degree of maturation of synapses. To conclude, TCP appears as a promising drug for the protection and long term survival of neurons.
The peptide neurotransmitter, substance P (SP), is regulated and distributed differently than coexisting transmitters in sympathetic neurons. Dopaminergic superior cervical ganglion (SCG) elevates levels of the peptide within the ganglion, but not in the nerve terminals or in target organs. Further, the cytotokine, interleukin-1β (IL-1β), stimulates levels of SP in mouse preoptic anterior hypothalamic mRNA in culture. These observations suggest that SP may help regulate intraorgan function, which in turn may be mediated by endogenous cytokines. To test this hypothesis, cultures of the SCG were treated with recombinant IL-1 receptor antagonist (IRLA) derived from activated human monocytes. ILIRA largely blocked the increase in SP normally seen in explants and dissociated neuronal cultures grown in the presence of ganglion nonneuronal cells. Levels of SP were the same as control values when excess IL-1β was added to ILIRA treated cultures, indicating both the specificity and lack of toxicity of ILIRA. Further, IL-1 β treatment did not inhibit the stimulation of the peptide by another SP-activating cytokine, D-factor. In conclusion, these results suggest that endogenous IL-1β plays a role in mediating the regulation of SP expression in the SCG and supports the hypothesis that the peptide is involved in ganglion responses to injury.

The effects of dietary N-3/N-6 ratio on brain development in mice of maternal dietary ratios of N-3/N-6 fatty acids (FA), indicated that increasing the N-3/N-6 ratio was associated with slightly smaller development in mice of maternal dietary ratios of N-3/N-6 fatty acids (FA), apart from a smaller litter size in ratio 1. Covariance analysis results indicate that the N-3/N-6 ratio of the phospholipids in the developing brain the rate of increase tended to decrease beyond 0.5 (phosphatidylcholine, PC) and 0.25 (phosphatidylethanolamine, PE), such that the localization of calbindin in brain revealed an increase in both the nuclear protein sedimented at ~4S on gradients which is consistent with the Mr of ~50,000 deduced from RAR DNA. The specific [3H]-RA binding activity in the nuclear fraction was saturable and of high affinity (Kd ~0.2 nM). The nuclear [3H]-RA binding protein bound to DNA-cellulose, an activity characteristic of other members of the steroid hormone receptor superfamily. Northern blotting experiments suggest that the nuclear protein represents a RAR of the a subtype. The expression of RARα mRNA was unaffected by treatment of cells with RA (100 nM). In contrast, mRNA for RARβ was initially absent but was induced within four hours after treatment of cells with RA. These results support the hypothesis that RAR's are involved in human NB cell differentiation.

The effects of dietary N-3/N-6 ration on brain development in the mouse were analyzed in a series of pairs of decreasing size. There were no group differences in speed of learning the pairs (trials to criterion) or in repeated errors (repeated errors/total errors x 100) while learning new pairs (fist 5 days with a new pair). However, when tasks were well-known (last 5 days to criterion), deficient monkeys made a higher % repeated errors (24.1 ± 3.3 vs 6.4 ± 0.7 for the controls: Mean ± SD, p<0.01). Such perseverative errors may reflect deficits in attention or memory, or difficulty in inhibiting a previous response.

601.4 COMBINED Mg2+ AND Ca2+ DEFICIENCY DURING FETAL AND POSTNATAL LIFE INCREASES LEVELS OF THE 28kDA CALCIUM-BINDING PROTEIN (CALBINDIN) IN BRAIN, S. Rose, B. Cheng, B. Rychlik and M. P. Matison. Center on Aging and Dept. of Anat. & Neurophysiology, University of Kentucky, Lexington, KY.

Rats were maintained on a diet deficient in Mg2+ (<0.003%) and Ca2+ (<0.02%), or a control diet (0.065% Mg2+ and 0.75% Ca2+), beginning on day 4 of pregnancy and through 3 weeks postpartum. Overall growth of pups from nonmaternal Mg2+/Ca2+ deficient diet was significantly retarded; in 2-week-old pups body and brain weights were reduced 40-60% and 20%, respectively. The pups of mothers on the Mg2+/Ca2+ deficient diet often died within 3-5 weeks of birth. Cell counts in hippocampi revealed a >40% reduction in neuronal density in regions CA1 and CA3, and a 20% reduction in neurite in CA1 and CA3; 50% of the pups of mothers on the Mg2+/Ca2+ deficient diet. Immunocytochemical localization of calbindin in brain revealed an increase in both the number of calbindin immunoreactive neurons, and in their relative staining intensities in both hippocampus and cerebral cortex. Western blots of whole brain demonstrated a large increase in calbindin levels in pups from mothers maintained on the Mg2+/Ca2+ deficient diet. In contrast, magnesium and calcium are known to impair cell function against excitotoxicity (Neuron, 6:41). The increased levels of calbindin in Mg2+/Ca2+ deficient pups may represent an attempt to compensate for the increased vulnerability to excitotoxic injury by maintaining neuronal calcium-binding protein levels. (Supported by NIH Grant NS 09678, NS07144 and HL18645. L.E.W. is an affiliate of the CMDRC.)


Platelet-derived growth factor (PDGF) was originally recognized as a major mitogen in human platelets. This growth factor exists as hetero- and homodimers of two distinct subunits (A and B) with PDGF-B being the major component of platelet granules. PDGF-B chain has been localized to specific neurons and fibers of the monkey CNS (Sasahara et al., Cell, 64, 1991).

We have examined the distribution of PDGF-B-chain-like immunoreactivity (PDGF-B-I) in the CNS of rats at various embryonic (E) and postnatal (PN) ages. At E14, PDGF-B-I is seen in specific developing fiber systems especially the main and accessory olfactory nerves (ON), but not generally with perikarya. However, distinct labeling of ON fibers persisted through all stages examined and into adulthood. This persistent and particularly heavy reactive may be related to the fact that ON neurons are continuously generated throughout life and suggest that PDGF may serve in some way to support the regenerating fibers. While many reactive cell bodies are visible in the periventricular proliferative zone at E14, reactive cells identifiable as neurons by their site and position are not apparent until PN 1.5 when lightly reactive pyramidal and Purkinji cells seen are in the developing hippocampus and cerebellum. The intensity of staining of these neurons as well as that certain other nuclei gradually increases with age. As the above findings suggest that PDGF may have roles in both the development and maintenance of the CNS, we are undertaking studies of the expression of PDGF-B-I in donor olfactory bulbs which have been transplanted to the site vacated by removal of a host ON. These studies along with general anatomical localization will be presented. (Supported by NIH Grant NS 09678, NS07144 and HL18645. L.E.W. is an affiliate of the CMDRC.)


A dose-response study was conducted to examine the effects on brain development in mice of maternal dietary ratios of n-3/n-6 fatty acids (FA), where n-3 FA were supplied as the preferred long-chain compounds. From conception until 12 days after birth, Beddf2 mice were fed dietary n-3/n-6 ratios of 0.2, 0.25, 0.5, 1.0, 2.0 and 4.0, with an n-6 content of greater than 0.5% energy in all diets, and similar levels of total polyunsaturated FA. Biochemical analyses were conducted on pup brains, as well as on samples of maternal milk. There were no obvious effects on overall pup growth and development, apart from a smaller litter size in ratio 1. Covariance analysis indicated that increasing the n-3/n-6 ratio was associated with slightly smaller brains, relative to body weight. Increasing dietary n-3/n-6 ratios generally resulted in a corresponding decrease in n-6 FA. The n-3/n-6 ratio of the milk related to that of the diet, but in the brain the rate of increase tended to decrease beyond 0.5 (phosphatidylcholine, PC) and 0.25 (phosphatidylethanolamine, PE), such that there was a significant quadratic relationship. These results indicate that the n-3/n-6 ratio of the phospholipids in the developing mouse brain respond maximally to maternal dietary long-chain n-3/n-6 ratios of between 0.25 and 0.5, which are equivalent to a n-3/n-6 ratio of approximately 0.25 in maternal milk.

Supported by a grant from NSERC (Canada).
601.7 
SENSITIVITY TO COCAINE IN RAT PUPS GIVEN PRENATAL COCAINE EXPOSURE. S. S. Meyer, J. D. Sherlock, and N. MacDonald.* Dept. of Psychology, Neurosciences, and Behavior Program, University of Massachusetts, Amherst, MA 01003. 
This study determined whether prenatal exposure to cocaine alters behavioral sensitivity to postnatal cocaine administration. Pregnant Sprague-Dawley rats were divided into three groups: Cocaine-treated, Saline-treated, and Untreated. The cocaine group was injected s.c. 2 days/day with 20 mg/kg cocaine HCI from gestational day 1 to day 20. Saline controls were pair-fed to the cocaine females, whereas untreated controls were undisturbed. Litters were culled to 8 pups (normally sex-balanced) and were fostered to normal lactating dams on postnatal day (PD) 1. On PD11, subjects were given either saline or cocaine (1.25, 2.5, or 5.0 mg/kg), returned to the nest for 15 min, and then removed for testing. Distress vocalizations and other behaviors were recorded during a 0-min test session. The results indicated that while acute cocaine treatment markedly suppressed distress vocalizations, there was no shift in the dose-response curve as a function of prenatal condition. In contrast, cocaine-induced activation of wall-climbing was influenced by prior cocaine exposure. Both control groups displayed a near-maximal increase in wall-climbing to the lowest cocaine dose, whereas the cocaine-pretreated pups showed little response except for the high (5.0 mg/kg) dose. Thus, prenatal cocaine treatment alters the sensitivity of some but not other behaviors to subsequent cocaine exposure. Supported by NIDA Grant DA-06495.

601.8 
The effects of maternal alcohol ingestion on ornithine decarboxylase (ODC) and organ weights were examined in 8-day-old rat pups. Control dams were fed a similar isocaloric diet maintained without sufficient water. Relative dehydration may thus lead to a decrease in the long-term trajectory of brain growth.

601.9 
EFFECT OF PRENATAL ETHANOL EXPOSURE ON ETHANOL TOLERANCE IN ADULT OFFSPRING IS DEPENDENT ON BLOOD LEVEL AFTER CHRONIC ETHANOL EXPOSURE. H. C. Becker and R. L. Haele, VA Medical Center and Medical University of South Carolina, Charleston, SC 29403. 
We previously found that prenatal ethanol (EtOH) exposure renders adult offspring less tolerant to the motor incoordinating effects of EtOH following chronic exposure to the drug. The purpose of this study was to examine whether this effect is influenced by the degree of chronic EtOH intoxication prior to tolerance testing. Female C3H mice derived from dams maintained on isocaloric liquid diets containing 8% or 8% EtOH-derived calories. Standard lab chow were used as subjects. Offspring were placed in inhalation chambers and exposed to either EtOH vapor or air for 4 hrs. Six hours following withdrawal, all mice were injected i.p. with 3 g/kg EtOH and tested for motor coordination on a horizontal dowel. Immediately after fall, a sample of cortex was collected. Results indicated that EtOH-exposed offspring fell off the dowel at significantly lower brain EtOH levels than control offspring following chronic exposure that yielded blood EtOH levels of 185 ± 3.7 mg% (i.e., prenatal EtOH offspring exhibited less tolerance). This prenatal treatment difference was not observed with chronic exposure that resulted in blood EtOH levels of 93.1 ± 0.7 mg%. Significant decreases in blood EtOH levels following chronic treatment were seen only in offspring with low prenatal EtOH exposure. These results demonstrate that, while there may be some rebound in neuronal functioning following alcohol exposure, the deficits induced in some neuronal complements are permanent.
EFFECTS OF TREATMENT OF PREGNANT ALCOHOLIC RATS WITH 8-OH-DPAT HAD NO SIGNIFICANT EFFECT ON 5-HT OR 5-HIAA LEVELS IN THESE TREATMENT GROUPS. ADDITIONAL STUDIES WILL DETERMINE IF PREGNANT ETHANOL TREATMENT RESULTS IN A SEROTONIN (5-HT) DEFICIENCY. BECAUSE FETAL 5-HT IS IMPORTANT FOR NORMAL CNS DEVELOPMENT, A 5-HT DEFICIENCY COULD CONTRIBUT TO THE ABERRANT MITOTIC ACTIVITY IN THE DEVELOPING TADPOLE CEREBELLUM FOLLOWING ETHANOL EXPOSURE.}

**EFFECTS OF EXPOSURE TO ETHANOL DURING DEVELOPMENT ON PLAY BEHAVIOR.**

M.L. Weaver*, C.S. Cretan*, D.A. Ciraulo*. Outpatient Clinic, Boston, MA.

These experiments demonstrated that the offspring of pregnant rats exposed to ethanol during the second half of gestation and the early postnatal period had a grossly simplified and abnormal dendritic tree. Neurons from animals exposed to the 20% EDC diet had a grossly simplified and abnormal dendritic tree. Neurons from the 3.2% EDC group exhibited stunted growth as demonstrated after the images were digitized and evaluated.

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MINIMAL REQUIREMENTS FOR LONG-TERM SERUM-FREE BIOELECTRIC ACTIVITY IN CULTURED CEREBRAL CORTEX. These cultures meet the following requirements: they are maintained in a serum-free medium; (2) the cultured cells form a near monolayer; (3) the media contain conditions that promote astroglial outgrowth and control on PD 10, via the high resolution in 3 dimensions provided by confocal microscopy. Pups for ex vivo studies were euthanized on PD 4. On PD 4-9, they were given 4.5 g/kg of alcohol per day (delivered in 2 of 12 daily feedings as a 50% v/v solution in milk formula; mean peak BAC=294 mg/dl), or were given a caloric-equivalent volume of bovine serum albumin (BSA). Pups were perfused on postnatal day 10, and 50-μm frozen sections were processed for GFAP immunoreactivity using a polyclonal antibody to GFAP. Matched sections from control and alcohol-exposed pups were evaluated with a Bio-Rad MRC 600 confocal microscope [60x objective, N.A. 1.4], sampling from at least 6 locations in layer V of parietal cortex in each section. Unbiased estimates of cell densities were determined using a 95 μm-square counting frame through a 2 x 10 μm slice (taken from 1 mm steps). The mean density of labeled astrocytes was significantly higher in alcohol-exposed tissue compared to controls, with an increase of 300% as result of a decrease of intracellular taurine as a consequence of taurine deprivation, and was not present when the antiserum was preabsorbed with taurine. The observed changes may be the consequence of cell swelling occurring as a result of a decrease of intracellular taurine as a large number of recent reports substantiate the involvement of taurine in osmoregulation in brain.
We are studying direct (extracellular) effects of light in the visible range on mammalian tissue. At intensities capable of penetrating the tissue, we have found that such light influences release of a neurotransmitter from mammalian brain tissue (Wade et al., PNAS 1988, 85:9353). At a certain intensity it also enhances [3H-2-deoxyglucose uptake into the tissue suggesting a possible effect on cell metabolism. Therefore, as a first step toward understanding what role light may have in cell metabolism, we used this light as a probe of metabolism and examined the extent of growth in several lighting conditions. This is based on studies in which supplemental environmental light increased body growth and body products (e.g., Am. J. Physiol. 1971, 220:233, 1975, 264:495, 1980, 239:491-93).

In the present study mice pups (strain Crl:CD-1ICR(BRR)) were reared with foster mothers from postnatal day 4 to 28 in either dark, 12h light/12h dark (standard), or 16h light/8h dark (supplemental). Details of the variation in intensity, duration and spectrum will be presented. Growth as measured by body weight (mean pup weight in grams ± S.E) was slow in the dark (e.g., at day 28, 11.37 ± 1.16 g, n=15), greater in the standard condition (15.06 ± 0.45, n=30), and greatest in the supplemental condition (16.87 ± 0.92, n=20). Feed efficiency of weight gain as defined by total weight gain of mother and pups per total food consumed during the experiment was greatest in the supplemental and least in the dark condition. The increase in growth induced by the light indicates an effect on metabolism; whether it is direct and/or indirect is yet to be determined.

Supported by NSF grant BNS-8702417 to P.D.W. & NIH training grant 5T32NS07034.

AGING PROCESSES V

602.1
A MAGNETIC RESONANCE IMAGING (MRI) STUDY OF BRAIN MORPHOMETRIC CHANGES WITH HEALTHY CHANGES IN HUMAN MALES. D. Murphy, C.J. DeCarli, W.S. Williams, S.J. Rapoport, M.B. Schapiro, B. Horwitz. National Institute on Aging, Bldg 10, Rm 6C 414 Bethesda, MD 20892.

Aging is associated with an increase in the prevalence of motor abnormalities. We wished to examine the effect of aging on the volume of cerebrospinal fluid (CSF) spaces, and the subcortical nuclei. We used MRI to determine the volumes (vol) of the cerebrospinal fluid (CSF), subthalamic nucleus, caudate nucleus, and ventricles. Measurements of 40 healthy normotensive, normolipidemic men and women age 20-50 (mean age 47.8 years; SD 17.4) and 10 female (mean age 47.8 years; range 20-79; SD = 20-47) subjects were neurologically normal, and free of structural lesions on MRI. Lesions were performed on a Picker 0.5 Tesla scanner. Axial slices (TR 2000 msec, TE 20 msec) with 192 views were analyzed, using a T1 (TR 2000 msec, TE 20 msec) sequence with 192 views and 2 repetitions. 18, 7mm thick, contiguous slices were obtained from the foramen magnum to the vertex. The results of the OM were compared to the YM with a t-test for differences between the groups and a Tukey's HSD post-hoc test for differences between the subcortical nuclei and the ventricles.

602.2

This investigation examined age-related changes in the basilar dendrites of pyramidal cells in Wernicke's area of left and right hemisphere. Tissue was obtained from 10 male (Mage = 52.2, range = 18-78, SD = 17.43) and 10 female (Mage = 47.8 years; range = 20-79; SD = 20-47) subjects. All subjects were neurologically normal and free of structural lesions on MRI. Lesions were performed on a Picker 0.5 Tesla scanner. Axial slices (TR 2000 msec, TE 20 msec) with 192 views were analyzed, using a T1 (TR 2000 msec, TE 20 msec) sequence with 192 views and 2 repetitions. 18, 7mm thick, contiguous slices were obtained from the foramen magnum to the vertex. The results of the OM were compared to the YM with a t-test for differences between the groups and a Tukey's HSD post-hoc test for differences between the subcortical nuclei and the ventricles.

602.3

We investigated age-related differences in cerebellar volumes. The areas of the three vermal regions were estimated from digitized midsagittal MRI scans of 29 healthy Ss and 24 neurologically intact Ss free of vestibular symptoms, seizures, psychosis or alcoholism (age 18-78). The three ROIs included: 1-centralis and culmen; 2-decciveum, folium, tuber, and pyramid; 3-uncula and nodulus. Ventral pons was used as a control region. After covarying cranial size, we found age-related decline in the vermis area (F (3, 47) = 4.15, p < 0.01). This difference was found only for the superior segments of the vermis (F (1, 49) = 4.45 and F (1, 49) = 6.75 for ROIs 1 and 2, respectively), but not for the uvula-nodulus (F < 1). Pons was unaffected by age. No sex differences were found. The observed vermal regions in the elderly were significantly smaller (p < 0.05) in the OM than the YM. In the OM the volume of the thalamus did not change with age. The volume of the thalamus was expressed as a ratio of CBM vol. The vol of the thalamus did not correlate with age.

602.4
DIFFERENTIAL CELLULAR CHANGES IN THE FRONTOPARietAL CORTEX AFTER BASAL FOREBRAIN LESIONS IN ADULT AND AGED RATS. C.U. Wellman and D.R. Sampels. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

Because aging can be viewed as a regressive process that alters both cell numbers and plasticity of remaining neurons, aging may interact with other degenerative processes to produce unique pathological syndromes. To understand the mechanisms of aging and disorders associated with aging, we are using basal forebrain lesions in adult and aged rats to determine the correlation and potential interaction of age and depletions of cholinergic input on frontoparietal morphology. Thirteen- and 21-month-old rats received either sham or basal forebrain lesions in the frontal cortex. In both adult and aged rats, lesioned rats, but not sham-lesioned rats, exhibited a significant decrease in cell size in the superficial laminae of the anterior cingulate gyrus. These changes in cell size were observed in both age groups, but were more pronounced in the 21-month-old rats compared to the 13-month-old rats. The pattern of changes also differed between the two ages: changes tended to be more pronounced in the superficial laminae of adult rats, whereas aged rats showed more changes in the deeper laminae. Thus, the magnitude and pattern of regressive changes in frontoparietal cortex after basal forebrain lesion differ in adult and aged rats, indicating that aging interacts with cholinergic depletion to produce unique neural changes.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
DEGENERATIVE CHANGES IN BASAL FOREBRAIN CHOLINERGIC NEURONS IN AGED RATS: A QUANTITATIVE ANALYSIS OF CELLS STAINED FOR CHAT OR NGF, OR RETROGRADELY LABELLED WITH FLUORESCENT TRACER OR I-125 NGF. S. de Lecea, J.D. Cooper, C.N. Svendsen, S.D. Dunnett and M.V. Sofroniew. Departments of Anatomy and Experimental Psychology, University of Cambridge, England. The effects of aging on basal forebrain cholinergic neurons were investigated in male Sprague-Dawley rats aged 3-6 or 24-33 months by immunohistochemical detection of CHAT or NGF, and by retrograde transport of Fluoro Gold (FG) or I-125 labelled NGF injected into multiple sites in the cerebral cortex and hippocampus. Cell counts and surface area measurements were prepared for precisely defined subdivisions of the basal forebrain cholinergic system: medial septum (MS), vertical and horizontal diagonal band, and basal nucleus (BN). Values from about one-third of the aged rats were not different from those of young adults; the remaining aged rats showed degenerative changes either in the form of cell loss or atrophy in several regions of the parameters tested. The degree of degeneration varied considerably among individual rats and among different regions, the MS being affected the least and the BN the most. Some aged rats showed a decline in the ratio of F.G./CHAT labelled cells, suggesting an impaired ability to retrogradely transport tracer, while others showed reduced retrograde labelling with I-125 NGF. These findings suggest that in some but not all aged rats basal forebrain cholinergic neurons decline in number and atrophy in the form of cell shrinkage, reduced CHAT immunoreactivity, and reduced capacity to retrogradely transport tracers and I-125 NGF.

THE EFFECTS OF LONGTERM VOLUNTARY ETHANOL CONSUMPTION ON THE AGING OF SYMPATHETIC NEURONS IN THE RAT. F.J. Jaatinen, A. Hervonen, H. Alho and K. Kiianmaa. 1 Laboratory of Gerontology, Dept. of Biophysics, University of Helsinki, Finland. 2 Department of Histology and Histotechnology, University of Tampere, Tampere, Finland. The effect of two years' voluntary ethanol intake (mean: 6.5 g/kg/day) on the aging of the nervous tissue was studied in alcohol preferring AA (Alko) rats, here we report the ethanol-induced changes in the aging of superior cervical ganglion (SCG) neurons. The initially 3-month-old rats were given access to food, tap water, and 10% ethanol solution (ethanol group) or to food and water only (control group). At 28 months of age the rats were killed, and the samples of the SCG were processed for histochemical, morphometric, and electron microscopic studies. The long-term ethanol exposure increased the heterogeneity of the aged neuron population and produced several signs of enhanced neuronal degeneration. The packing density of SCG-neurons (the number of neurons with a large nuclear profile per 90.000μm²) decreased from 42.7±0.97 (mean±SEM) at the age of 3 months to 25.5±1.34 in the controls and 21.0±3.13 in the ethanol group at 28 months. The amount of highly pigmented neurons was 39.3±2.5% in the old control rats and 59.3±4.2% in the old ethanol group. The overall intensity of TH-immunoreactivity and catecholamine histochemistry was also lower in the ethanol rats than in the controls. The density of fluorescence intensity of the peripheral neuronal network in the superior cervical ganglion (SCG) was decreased in the ethanol-exposed rats. The monoamine contents of different brain areas did not change due to ethanol exposure. The survival of ethanol and control rats did not differ either. Thus the results may indicate a selective vulnerability of sympathetic neurons rather than a universal accelerated aging due to ethanol exposure.

INCREASING NEURON NUMBER WITH BODY SIZE: A TEST OF THE SELECTION HYPOTHESIS. P.G.R. Stecker and P.H. Patel. Dept. of Physiology, Univ. N. Car. at Chapel Hill, NC 27599. Large bullfrogs have more neurons in the hindlimb motor pool (JCN 261:125, 1966) and in dorsal root and sympathetic ganglia (Soc. Neurosci. Abstr. 15:301, 1989) than small frogs. The possibility that this association is due to counting error was eliminated by 3-D reconstructions of HRP-labelled neurons, which showed that nuclei are not split in sectioning. A second alternative to neuron addition is that, by selection, only those frogs with many neurons survive to retain large size. Thus, the difference in mean number of neurons between small and large animals may be due to a dropping out over time of those animals with few neurons. This possibility can be tested by counting neurons from the same closed population at different points in time. Twelve size-matched frogs were randomly divided into two groups shortly after metamorphosis. Six juvenile frogs, with a mean length of 4.0±1.1 cm (mean±SEM) and a mean weight of 5.6±2.2 g, were killed immediately. At the end of one year, the remaining six animals (mean length 6.7±2 cm, mean weight 26.6±2.4 g) had substantially more lumbar sympathetic neurons (7756±571 vs 3398±413) and dorsal root ganglion neurons (9930±572 vs 5065±449). These data show that selection cannot account for the relationship between body size and neuron number. Previous studies of H-thymidine incorporation provided no evidence for neuron birth after metamorphosis. The explanation most consistent with these results is that immature nerve cells differentiate with increasing body size.

THE EFFECTS OF LONGTERM VOLUNTARY ETHANOL CONSUMPTION ON THE AGING OF SYMPATHETIC NEURONS IN THE RAT. P.J. Jaatinen, A. Hervonen, H. Alho and K. Kiianmaa. Laboratory of Gerontology, University of Tampere Medical School, Tampere, and Research Laboratory Ltd., Helsinki, Finland. The effect of two years' voluntary ethanol intake (mean: 6.5 g/kg/day) on the aging of the nervous tissue was studied in alcohol preferring AA (Alko) rats, here we report the ethanol-induced changes in the aging of superior cervical ganglion (SCG) neurons. The initially 3-month-old rats were given access to food, tap water, and 10% ethanol solution (ethanol group) or to food and water only (control group). At 28 months of age the rats were killed, and the samples of the SCG were processed for histochemical, morphometric, and electron microscopic studies. The long-term ethanol exposure increased the heterogeneity of the aged neuron population and produced several signs of enhanced neuronal degeneration. The packing density of SCG-neurons (the number of neurons with a large nuclear profile per 90,000μm²) decreased from 42.7±0.97 (mean±SEM) at the age of 3 months to 25.5±1.34 in the controls and 21.0±3.13 in the ethanol group at 28 months. The amount of highly pigmented neurons was 39.3±2.5% in the old control rats and 59.3±4.2% in the old ethanol group. The overall intensity of TH-immunoreactivity and catecholamine histochemistry was also lower in the ethanol rats than in the controls. The density of fluorescence intensity of the peripheral neuronal network in the superior cervical ganglion (SCG) was decreased in the ethanol-exposed rats. The monoamine contents of different brain areas did not change due to ethanol exposure. The survival of ethanol and control rats did not differ either. Thus the results may indicate a selective vulnerability of sympathetic neurons rather than a universal accelerated aging due to ethanol exposure.

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INCREASING NEURON NUMBER WITH BODY SIZE: A TEST OF THE SELECTION HYPOTHESIS. P.G.R. Stecker and P.H. Patel. Dept. of Physiology, Univ. N. Car. at Chapel Hill, NC 27599. Large bullfrogs have more neurons in the hindlimb motor pool (JCN 261:125, 1966) and in dorsal root and sympathetic ganglia (Soc. Neurosci. Abstr. 15:301, 1989) than small frogs. The possibility that this association is due to counting error was eliminated by 3-D reconstructions of HRP-labelled neurons, which showed that nuclei are not split in sectioning. A second alternative to neuron addition is that, by selection, only those frogs with many neurons survive to retain large size. Thus, the difference in mean number of neurons between small and large animals may be due to a dropping out over time of those animals with few neurons. This possibility can be tested by counting neurons from the same closed population at different points in time. Twelve size-matched frogs were randomly divided into two groups shortly after metamorphosis. Six juvenile frogs, with a mean length of 4.0±1.1 cm (mean±SEM) and a mean weight of 5.6±2.2 g, were killed immediately. At the end of one year, the remaining six animals (mean length 6.7±2 cm, mean weight 26.6±2.4 g) had substantially more lumbar sympathetic neurons (7756±571 vs 3398±413) and dorsal root ganglion neurons (9930±572 vs 5065±449). These data show that selection cannot account for the relationship between body size and neuron number. Previous studies of H-thymidine incorporation provided no evidence for neuron birth after metamorphosis. The explanation most consistent with these results is that immature nerve cells differentiate with increasing body size.
503.1 MODIFICATION OF NON-ROENTGEN DETECTION OF NEUROTRANSMITTERS BY IN SITU HYBRIDIZATION IN THE RAT BRAIN. N. Nourse-Gohl*, T.C. Cavanagh* and D.S. Gage*. R&D Division, Boehringer Mannheim Corp. and Indiana Univ. Sch. of Med., Program in Medical Neurobiology, Indianapolis, IN 46250.

In situ hybridization histochemistry is a powerful tool to localize specific mRNAs to individual cells. This is especially important in the nervous system where the complex structural interactions of diverse neuronal and glial cell types need to be preserved. Non- roentgen detection of mRNA hybridization will allow quantitative analyses of primary Ab binding kinetics in future studies. Supported by AG-7892, AHA-GIA 891079, and AHA-EIA 890173.


These results support the validity of the radioimmunohistochemical method as a tool for quantifying proteins at cellular level and confirm that TH protein content is differentially regulated in noradrenergic and dopaminergic neurons in response to reserpine.


Most of the immunohistochemical procedures developed for this purpose, we therefore developed a rapid and sensitive radioimmunohistochemical method for tyrosine hydroxylase (TH) quantification in fresh-frozen rat brain. The present study was performed in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), containing dopaminergic neurons and the locus coeruleus (LC) and subcoeruleus (SuCh), containing noradrenergic neurons, of rats treated with reserpine. Autoradiography of TH was performed on TH coronal sections from unfixed brain of rats treated either with reserpine (10 mg/kg) or vehicle. Sections were incubated with TH monoclonal antibody for 30 min and the reaction was revealed with a peroxidase secondary antibody. TH was quantified in the different brain regions by measuring optical density (OD) on film or emission autoradiography and compared with adequate TH standards, using computer-assisted image analysis. TH concentrations (expressed in ng/mg prot) measured on films were 40% increased by reserpine in the LC and 20% in the SuCh but were unchanged in the SNc and in the VTA. Neuroanatomical localization of TH radioimmunolabeling on emission-coated sections showed a heavy labeling appearing as an accumulation of silver grains over cell bodies and also over processes. Grain density per cell, considered as representative of the amount of TH on individual cells, was increased in the LC and subCh but not in the SNc. These results support the validity of the radioimmunohistochemical method for TH quantifying proteins at cellular level and confirm that TH protein content is differentially regulated in noradrenergic and dopaminergic neurons in response to reserpine.


In brain sections that have been stored for several years in cryoprotectant solution, this protocol is suitable for the localization of rat, mouse, monkey, and possibly human. Supported by NICHD MH 45573.

503.5 KINETIC ANALYSIS OF SECONDARY ANTIBODY BINDING TO FLOATING BRAIN SECTIONS. A NEW METHOD FOR QUANTITATIVE IMMUNOCYTOCHEMISTRY. D.G. Morgan, D.G. Buxton and M.N. Gordon. Div. of Neurogerontology, Univ. of Southern California, Los Angeles, CA 90089-0191.

The binding of a secondary (2°) antibody was prevented by diluting antisera in 0.1% bovine serum albumin (BSA). Dilution of antisera in 0.1% BSA did not reduce the binding of biotinylated anti-sheep secondary antibody to sheep IgG. This complex is visualized using an avidin-biotinylated peroxidase or alkaline phosphatase detection system. This detection method results in a reaction product which is more immunocytchemically desirable.

This report describes the modification of the detection method for localizing digoxigenin-labeled oligonucleotide probes used for in situ hybridization in the rat nervous system. Ordinarily following hybridization with cellular mRNA, the 3'-tailed oligo probe is localized using an antibody covalently attached to a fluorophore and a subsequent color reaction. In the present study, whole anti-digoxigenin sheep polyclonal antibody is used as a primary antibody, followed by a biotinylated anti-sheep secondary. This complex is visualized using an avidin-biotinylated peroxidase or alkaline phosphatase detection system. This detection method results in a reaction product which is more immunocytchemically desirable.

In this study the expression of 5-hydroxytryptamine (5-HT) and peptide mRNA, we have developed a procedure combining immunocytochemistry with in situ hybridization using 35S-labelled oligonucleotides. Peroxidase anti-peroxidase immunocytochemistry and immunofluorescence were tested and were applied either preceding or following in situ hybridization. Combined 5-HT immunocytochemistry and peptide in situ hybridization was obtained using immunofluorescence followed by in situ hybridization. The fluorescence survived a post-hybridization 5% wash and liquid emulsion autoradiography. Loss of mRNA during immunostaining was prevented by diluting antisera in 0.1% BSA.

The amount of probe used for each section was 50,000 (rat) and 500,000 (monkey).

Using this protocol, the results in rats showed better specific labeling with less nonspecific background compared to controls. Excellent resolution was obtained from monkey sections that were stored in cryoprotectant for 31 months. As with conventional in situ hybridization methodology, the prehybridization treatment step is not necessary to reduce the background. This protocol is an efficient procedure to allow the detailed labelling of brain sections that have been stored for several years in cryoprotectant solution.
INTRACELLULAR INJECTIONS OF BIOTINYLATED LUCIFER YELLOW LOW IN ELECTRON MICROSCOPIC STUDIES: A COMBINED EM AND IMMUNOCYTOCHEMICAL LABELING TECHNIQUE. G. S. Hill and D. L. Oliver, Dept. Anatomy, UCCONN Health Center, Farmington, CT 06032

Intracellular injections have been used extensively to study the fine structure of nervous cells. In the present study, we report on the use of Lucifer Yellow CH in combination with immunocytochemistry for the study of the fine structure of neurons and their dendrites.

603.7

INTRACELLULAR INJECTIONS OF BIOTINYLATED LUCIFER YELLOW LOW IN ELECTRON MICROSCOPIC STUDIES: A COMBINED EM AND IMMUNOCYTOCHEMICAL LABELING TECHNIQUE. G. S. Hill and D. L. Oliver, Dept. Anatomy, UCCONN Health Center, Farmington, CT 06032

Intracellular injections have been used extensively to study the fine structure of nervous cells. In the present study, we report on the use of Lucifer Yellow CH in combination with immunocytochemistry for the study of the fine structure of neurons and their dendrites.

603.9

THE USE OF NICKEL IONS FOR TRACING CENTRAL CONNECTIONS AND PERIPHERAL NEURONS OF THE LICHEN NERVOUS SYSTEM. M. Nlabach and B. Macagn, Dept. of Biological Sciences, Columbia University, NY, NY 10027

We report here on the use of Ni²⁺ and Ni-labeled (Ni-Ly) as dyes both for nerve backfilling and for direct injection of identified neurons in the leech, Hirudo medicinalis. The male sex nerve was backfilled with either 5% NiCl₂ or 250mM NiCl₂. After precipitation of NiS and washing, the Ni-Ly was filled into the male sex nerve in several cases with 30% horseradish peroxidase (HRP). We always saw more backfilled neurons with Ni²⁺ or Ni-Ly than with HRP. This suggests that these dyes are more sensitive for backfills than HRP.

603.10

BIOTINYLATED DEXTRAN AMINE AS AN ANTEROGRADE TRACER FOR SINGLE- AND DOUBLE-LABELED STUDIES. A. Renter, C. Veneman and M.G. Hong, Department of Anatomy and Neurobiology, University of Tennessee - Memphis, TN 38163

We report here on the use of biotinylated dextran amine (BDA) as an anterograde tracer for single and double-labeled studies. BDA was visualized with an avidin-biotinylated HRP (ABC) procedure followed by a DAB reaction. After iotophoretic injections of BDA into isocortex-like telencephalic regions in pigeons or into visual or somatosensory cortex in rats, we found excellent and abundant labeling of terminals in forebrain, midbrain and hindbrain target areas with one week survival times. Pressure injections of BDA into the ventral horn of embryonic chick spinal cord yielded extensive anterograde labeling of motoneuron axons in peripheral nerves. We also found that BDA labeling could be combined with a second labeling method to differentially label and distinguish two sets of projections. For these experiments, motoneuron and sensory neuron projections into the embryonic chick hindlimb were differentially labeled by HRP injection into the DRGs (visualized using a blue cobalt-DAB reaction) and BDA injection into the spinal cord (visualized with a brown DAB reaction). Taken together, these various studies show that BDA is effective for anterograde pathway tracing and can be used in double-label studies with other labeling methods. Supported by NS-19620 and NS-28721 (AR) and NS-26386 (MGH).

603.11


Normal rabbit serum (NRS) was pressure injected into the forebrain of rats to be tested as an anterograde neuroanatomical tracing substance.

603.12

THE USE OF HERPES SIMPLEX VIRUS TYPE 1 FOR NEURAL PATHWAY MAPPING IN MICE. S.A. Leonard, P.P. Sarna and F.F. Bloom, Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Herpes simplex virus type 1 (HSV–1) was used as a transneuronal pathway tracer in the CNS. HSV–1 was stereotaxically placed in the caudate putamen of mice and after varying survival times was visualized with a polyclonal antibody to HSV–1 proteins, and an antibody to mouse macrophage (Mac–1) with peroxidase-fractional fixative. Viruses was detected in brain sections by immunohistochemistry with a polyclonal antibody to HSV–1 proteins, and an antibody to mouse macrophage (Mac–1) was used to detect immune responses within the same sections. The retrograde, anterograde and transsynaptic transport of the virus was observed. HSV–1 was transported from the caudate along known major pathways and was detected in regions of the brain including the cortex, globus pallidus, substantia nigra, dorsal raphe nucleus, ventral tegmental area, and lateral habenula. The immunostaining patterns suggest that the virus then transported transsynaptically from these regions. The same–staining of the same sections revealed that activation of macrophages follows the same inter-neuronal pathways of the viral infection in a temporal manner.
603.13


In animal models, spread of HSV-1 from epithelial replication sites to the PNS and CNS is known from analysis of individual tissues. To more completely define tissue targets of infection and infer routes of spread, corneas of adult mice were inoculated with 10^7 pfu of HSV-1, strain F. One to 13 days later, groups of 4 mice were formalin-perfused. Decalcified head and neck were embedded in block in paraffin. At intervals, serial sections were screened for HSV antigen. On days 1 and 2, antigen was limited to cornea and conjunctiva but by days 3 and 4 was also in autonomic ganglia and the trigeminal system. On day 6, antigen reached its maximum extent; infected sites included the trigeminal complex (gaglion, ophthalmic and maxillary branches, root and spinal nucleus and tract), ethmoid sinus and olfactory bulb, visual system, and autonomic ganglia (ciliary, pterygopalatine and superior cervical). By days 8 and 10, antigen was greatly reduced, and day 13 samples were negative. This method shows a broader range of infected tissues and suggests a more complex pattern of HSV spread than has been recognized. Virus appears to reach the intracranial compartment by 4 different neural routes. Inoculum dose may alter the kinetics of virus spread to neural tissues.

603.14

TRACING OLFACTORY PATHWAYS IN THE MOUSE WITH A CORONAVIRUS. E.M. Barnett, M. Cassell and S. Perlman. University of Iowa College of Medicine, Iowa City, IA 52242.

Olfactory system pathways in the mouse were studied after intranasal inoculation of Mouse Hepatitis Virus (MHV) strain JHM, a neurotropic murine Coronavirus which is non-pathogenic to humans. Animals were sacrificed 4 and 5 days post inoculation and viral RNA was detected by means of in situ hybridization with a 35S labeled probe. Labeling was seen in the main olfactory bulb and its afferent and efferent connections including: the anterior olfactory nucleus, insular cortex, piriform cortex, the nucleus of the diagonal band, lateral hypothalamus, the nucleus of the lateral olfactory tract, and midbrain raphe nuclei. These results are consistent with transneuronal retrograde, and possibly anterograde, movement of virus. Viral RNA was also labeled in structures such as the endopiriform nucleus, the external lateral parabrachial nucleus, the ventral tegmental area, and medullary raphe nuclei suggesting viral movement across several synapses. These findings suggest that MHV strain JHM may be a useful neural systems tracer in the mouse.

603.15

β-GALACTOSIDASE EXPRESSING RECOMBINANT PSEUDORABIES VIRUS FOR LIGHT AND ELECTRON MICROSCOPIC STUDY OF RETROGRADE TRANSNEURONALLY LABELED CNS NEURONS. A.D. Loewy, P.C. Bridgman & T.C. Mettenleiter. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 and Federal Research Center for Virus Diseases of Animals, D-7400 Tübingen, Germany.

Bartha's strain of pseudorabies virus (Bartha PRV) is a neurotropic virus used in veterinary medicine to vaccinate pigs against Aujeszky's disease (i.e., the "mad itch"). It has also been shown to be a highly specific retrograde transneuronal cell body marker (Brain Res. 491:274, 1989 and J. Neurosci. 10:2139, 1990). The latter property makes this particular virus a potentially useful tool for neuroanatomy and other neurobiological studies.

A Bartha-galactosidase expressing Bartha PRV was constructed and used to transneuronally label the CNS autonomic neurons in rats that project to the sympathoadrenal preganglionic neurons and to the eye. Four days following injections of the β-galactosidase expressing Bartha PRV, virally infected nuclei were visualized with a one step histochemical reaction using the Bluo-Gal substrate (halogenated indolyl-β-D-galactoside). In some infected neurons, a Golgi-like staining of the axonal nuclei. These results are consistent with replication of Bartha PRV post infection into the CNS. Immunohistochemical reactions using the Bluo-Gal substrate (halogenated indolyl-β-D-galactoside) and high magnification. This virus may be useful for the study of the cell architecture and synaptic organization of transneuronally labeled neurons of functionally defined neural circuits. These results also demonstrate that it is possible to deliver foreign genes into specific chains of neurons in the mammalian CNS by means of the retrograde transneuronal viral labeling method.

603.16


Cajal was born in a humble room in a stone house in the village of Petilla, in the mountainous Aragon region of Northern Spain. Medical school was attended at the insistence of his father; later, Cajal began study and research in histology at the University of Zaragoza while convalescing after military service in Cuba. An appointment to the chair of anatomy at Valencia at age 32 was followed three years later by a move to the chair of histology at Barcelona. The next year, 1888, saw the beginning of his experimentation with the reduced silver method for staining the nervous system, introduced by Golgi. Cajal's facility with this and subsequent techniques employed from 1890 at the University of Madrid contributed to the revelation and faithful documentation of the histological organization of the central nervous system. The published documentation of this work reflects both technical and artistic accomplishment. Indeed, Cajal's early devotion to drawing and painting appear to have become submerged in his later career, in the preparation of scientific illustrations. The quantity and quality of Cajal's hand-prepared drawings will be considered, with a comparison to the talents and achievements of his contemporaries. They will also be placed in perspective to the origins and continuing development of techniques for the transcription, enlargement, and reproduction of hard copy histology.

603.17


Cajal and Golgi shared the Nobel Prize in Physiology or Medicine in 1906 in recognition of their work on the structure of the nervous system. Cajal's drawings of nerve cells, stained by his own and by Golgi's techniques, are marvels of precision and art. The Spanish government belatedly facilitated the achievements of the largely self-taught professors of anatomy at the University of Madrid by providing a new building in 1932. The top floor was for the master's microscopic research and the remainder accommodated programs in subspecialties beyond neuroscience, this building was completed much earlier in Vienna, Leipzig, and Zurich. With the new facilities, Cajal continued to expand a school of followers, including Lorente de Nó and Rio-Ortega. His clear proof of collateral sprouting after nerve injury and his detailed work on the development of neurons have earned him lasting places of discovery of reorganization of pathways in cerebral and cerebellar cortex. Cajal's emphasis on the synapse, fundamental to his demonstration of the correctness of the neuron doctrine, further integrated his work and opened the central nervous system to new understanding. A beautiful modern institute now carries forward the elegant Spanish tradition of nervous system research.
604.1 THE GENERATION OF A MOUSE MUTANT FOR THE ALPHA SUBUNIT OF THE CA/CALMODULIN KINASE II THROUGH GENE TARGETING IN EMBRYONIC STEM CELLS. Alcino J. Silva*, Jeanne M. Wehner*, and Susumu Tonegawa*. * Cancer Center, Massachusetts General Hospital, Harvard Medical School, and MRC Laboratory of Molecular Biology, Cambridge, UK. ** Institute of Genetics, University of Colorado, Boulder, CO.

The brain-specific Ca/Calmodulin Kinase II (CamKII) is thought to have a role in a myriad of phenomena ranging from synaptic transmission to neural development. In the hippocampus and forebrain, two regions of the CNS often implicated in memory, this enzyme is mostly composed of alpha subunits. Using homologous recombination, we disrupted the alpha-subunit gene of CAMKKII in embryonic stem cells, and these cells were used to derive a mouse mutant for this gene. We will provide a preliminary report on our attempts to test these mice for abnormalities in development and long-term potentiation, for anatomical defects in structures such as the barrel fields of the somatosensory cortex, and for spatial memory deficits in a modified Morris water maze.


The synapsin I gene encodes a neuron-specific protein found in virtually all nerve cells. In order to define DNA sequences necessary for tissue-specific expression of this X-linked gene, we have cloned a 1.7 kb fragment of the synapsin I promoter region which encodes the 5' 150 bases of the gene. A line of transgenic mice was produced in which 10 to 20 copies of this transgene have integrated into an autosomal site. X-gal histochemistry stains most neurons, and the relative intensity of staining varies among neuronal cell types in a pattern similar to that observed for synapsin I mRNA in situ hybridization in the terminal fields. X-gal staining is high in cerebellum, olfactory bulb, dentate gyrus, hypothalamus and layers II, V and VI of cortex, and low in hippocampal interneurons. An unexplained observation is the near absence of staining in striatum. Furthermore, the amount of transgene mRNA in the brain is much lower than that of endogenous synapsin I mRNA. One possible reason is that the DNA sequences required for neuron-specific expression of the synapsin I gene are present in our construct. Nonetheless, the relatively low levels of transgene mRNA and protein suggest that additional regulatory sequences may be present so that position effects of the site of integration may affect expression, or that transgene mRNA stability may be different than that of the endogenous synapsin I mRNA. Supported by NS08591, NS18381 to KH, NS25050 to LD.

604.4 CHARACTERIZATION OF A PHOSPHOENOLPYRUVATE CARBOXYLASE PROMOTER SEGMENT CONFERS TRANS-SYNAPTIC REGULATION TO THE HUMAN PHENOMALPHA PROMOTER IN TRANSGENIC MICE. M. A. Goodall(1), A. C. Peterson(2), G. A. Rouleau(1) and T. P. C. Morgan(1). (1) The Montreal General Hospital Research Institute, Canada. H3G 1A4. (2) Ludwig Institute for Cancer Research, Montreal, H3A 1A1.

Neurofilaments (NFs) are the most abundant cytoskeletal elements of neurons. They are formed by the co-polymerization of three neurofilament proteins, NF-L, NF-M and NF-H. NF-H is believed to form the side arm projections seen in NFs. Various observations suggest that neurofilaments may exist as a centrosomal unit, i.e., for NFs, in the maintenance of neuronal calibre. To further study the role of NF-H, we have generated a transgenic mouse expressing the human NF-H gene (hNF-H). A complete genomic clone for the human NF-H gene has been obtained by screening a cosmId library using the hMN-1 restriction enzyme and microinjected into mouse eggs. Two founder mice have been generated with a copy number of two copies of the hNF-H gene have been obtained. Northern blot analysis of total RNA from various tissues indicates that the human gene is specifically expressed in the mouse nervous system. Work is in progress to determine the effect of high expression of the hNF-H protein on the axonal NF architecture.
604.7
CHARACTERIZATION OF PROENKEPHALIN HETEROCUGUON RNA IN INDIVIDUAL NUCLEI IN THE RAT BRAIN. Marlin K.-H. Schaller, T.-Y. Fang Liu* and S.L. Zipf. Mental Health Research Institute and Dept. Physiology, University of Michigan, Ann Arbor, MI 48191 USA
While in vitro transcrption assays have proven to be a powerful method to assess the transcriptional activity of specific genes, their application to brain tissue has been limited. An alternative approach involves analyzing changes of steady state levels of primary RNA transcripts of specific genes, which are rapidly processed in the cell nucleus by intron splicing into mature mRNA. Using probes specific for the short-lived intervening sequences, primary RNA transcripts and processing intermediaries can both be localized in situ. Intransons are characterized by differential sensitivity in RNA protection assays. The goal of this study has been 1) to generate specific tools for proenkephalin (PENK) RNA transcripts to examine the cellular distribution of these RNA species in rat brain and 2) to establish the fundamental methodology to record rapid transcriptional changes of the PENK gene. A PENK genomic clone was isolated from a rat genomic library and DNA fragments containing either exon A or exon B sequences were subcloned into pGEM4 vectors to generate intron specific cRNA probes. Use of these intron specific 5'S-NTP labeled cRNA probes in an in-situ hybridisation assay of frozen brain sections revealed many positively labeled neurons in various brain areas after exposure time less than four weeks. The strongest signal occurred in striatum, in which silver grains were specifically localized over counterstained cell nuclei. These nuclei were located within PENK synthesizing neurons, as identified by labeling of adjacent sections with a PENK RNA probe specific for exon 3 of PENK mRNA. This probe yielded an identical labeling pattern, but with cytoplasmic localization of the grains. PENK in situ levels in brain regions of known lower abundance of PENK mRNA were below our detection limit. The existence of four mRNA intermediates and the splicing order of PENK in RNA is currently being assessed in RNA protection assays. Supported by NDA #DAI2065.

604.9
DIFFERENTIAL EXPRESSION OF CEREBELLIN-RELATED TRANSCRIPTS IN THE ADULT AND DEVELOPING RODENT NERVOUS SYSTEM. R. Kaskel*, Y. Uraga*, J. Ogishita*, R. Molinar-Rode* and J. Morgan. Department of Neuroscience, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110. To investigate the molecular genetic basis of cell-specific gene expression in the developing nervous system a series of genes have been cloned that show differential expression in cerebellum. Previous studies have established that a hexadecapeptide, termed cerebellin, is highly enriched in cerebellum. Furthermore, a number of cerebellar mutant mice have been generated with reduced levels of cerebellin. To pursue the question of the regulation of this neuropeptide we have cloned a cDNA and the cognate gene that encode the cerebellin sequence in the brain. To examine its gene structure and expression we have cloned a related gene, here termed pseudocerebellin, which has been used as a probe for transcription from whole brain mRNA. The protein encoded by this sequence contains the cerebellin motif with one amino acid change. This transcript appears to be more ubiquitously expressed in the CNS than cerebellin. We are examining the 5' regulatory sequences of the two genes to determine the basis of their differential expression using transgenic mouse technology.

604.10
ISOLATION AND EXPRESSION OF TRK PROTO-ONCOGENE FAMILY MEMBERS. D.S. Battleman*, C. Lai, D.A. Fishman, M.Y. Chao* and G. Lemke. The Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York 10021 and The Salk Institute, P.O. Box 10350, San Diego, CA 92186. The product of the trk proto-oncogene is a low affinity receptor for nerve growth factor (NGF) that participates in the formation of the high affinity NGF receptor complex (Hempstead et al., Nature 350, 678, 1991). Trk encodes a receptor tyrosine kinase which, together with p75 NGF receptor is responsible for a signal transduction by NGF. A direct prediction from these results is that the specificity of action of other neurotrophic factors is mediated by the expression of other trk-related receptor tyrosine kinases. We have utilized a PCR-generated fragment, tso-10 (Lai and Lemke, Neuron, in press) as a probe for trk-related tyrosine kinases. A 1.75 kb cDNA clone was isolated from a two week rat brain cDNA library. Northern blot analysis with this isolate revealed an expression pattern distinctly different from both trk and trk II. PC12 cells displayed as many as four distinct mRNA sizes, ranging in size from 3.5 kb to 7.7 kb. In developing rat brain only two transcripts of 4.2 kb and 7.7 kb size could be detected, with the 4.2 kb mRNA being the most prominent. Expression of this 4.2 kb transcript appeared to increase during embryonic and early postnatal period and declined after postnatal day fourteen.

604.11
DEVELOPMENTAL REGULATION OF NCAM, N-CADHERIN AND ACETYLCHOLINE RECEPTOR mRNA LEVELS IN CHICK SKELETAL MUSCLE. J. Capasso, C.G. Hahn, M. Sasner, S. Tilney and J. Coyault. MUSCLE. J. Capasso, C.G. Hahn, M. Sasner, S. Tilney and J. Coyault. Department of Physiology & Neurobiology, Univ. of CT, Storrs, CT 06269 (Supported by grants from the March of Dimes Foundation and the NIH # NS25264 and NS10338.)

604.12
DIFFERENTIAL EXPRESSION OF THE ANDROGEN-BINDING PROTEIN GENE IN THE RAT BRAIN. Y.M. Vang, P.M. Sullivan*, and P.R. Josephs. Curriculum in Neurobiology, Departments of Pediatrics and Biology, The Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, NC 27599. The observation that the androgen-binding protein (ABP) gene is expressed and processed differently in the rat brain (Endocrinology 132: 3124, 1990) led us to further examine its gene structure. A 20-kb fragment upstream from the testis specific exons was cloned and characterized. Three brain specific splicing regions were localized on this fragment by restriction mapping and sequencing. They are approximately 15, 6.3 and 4.8-kb upstream of testicular exon 1. One kb upstream of the 5'-most exon was located at the promoter and regulatory regions. Analysis of the sequence shows that it is extremely G-C rich with seven GC box-like elements. Sequences homologous with two AP2 binding sites, a CRE core sequence and an SHE were also found. Further study is underway to confirm the functional relevance of the above entities and to dissect the heterogeneity of ABP transcripts in the brain. (Supported by NIH grants HD-21744 and HD-18968).
604.13

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis and is regulated in a tissue-specific manner at the transcriptional level. Using site-directed mutagenesis, we have determined that the DNA segment between -205 and -182 bp from the transcriptional start site constitutes the minimal enhancer for the tissue-specific expression of the rat TH gene in cultured pheochromocytoma cells. This segment is composed of an AP1 motif and an overlapping 20 bp dyad whose core resembles an E box consensus site (TGATTCAGAGGCAGGTGCCTGTGA). Despite the overlap, both the AP1 and the dyad elements bind proteins independently in vitro. Interaction between the two elements, however, is necessary for in vivo expression. Therefore, we conclude that the synergism between the AP1 and dyad elements is responsible for the tissue-specificity of TH. This is the first identification of a tissue-specific element in a mammalian neuronal gene, and the first known case where tissue-specificity is elicited by an obligate synergy with an AP1 motif.

Despite the 1 nucleotide difference from the consensus, the AP1 motif in TH functions as a ubiquitous activator, and mediates cAMP response upon induction with forskolin and 8-bromo cAMP. The response to elevated level of cAMP by the AP1 motif is weaker than that via the cAMP response element (CRE) located downstream at -45. Unlike other neuroendocrine genes, the CRE in TH is not essential for the TH-induced genes. The CRE in TH, however, segments the basal level of TH transcription directed by the AP1 and E-box/dyad motif.

604.15

The helix-loop-helix (HLH) transcriptional factors play important roles in specifying different cell types in Drosophila neurogenesis and also in muscle differentiation of vertebrates. Using PCR and low stringency screening of cDNA libraries from chick embryonic neural tube from different stages of development, several cDNAs were isolated that have homology to the HLH region of the mouse and human E12/E47 transcriptional factor. Northern blot analyses with isolated that have homology to the HLH region of the mouse and human E12/E47 transcriptional factor. Northern blot analyses with isolated that have homology to the HLH region of the mouse and human E12/E47 transcriptional factor. Northern blot analyses with isolated that have homology to the HLH region of the mouse and human E12/E47 transcriptional factor. Northern blot analyses with isolated that have homology to the HLH region of the mouse and human E12/E47 transcriptional factor. 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605.4
ELECTROTROTONIC LENGTH ESTIMATES IN NEURONS WITH SOMA SHUNTING
W.J. Holmes and W.R. Rall. Neurobiology Program. Department of Zoological &
Biomedical Sciences, Ohio University, Athens, OH 45701 and Math. Research
Branch, NIDDK, NIH, Bethesda, MD 20892.

Previously (Soc. Neurosci. Abstr. 13:1517, 1987) we discussed the
discrepancies to be expected when equivalent cylinder formulae are applied
to neurons with soma shunting having different L values. Here we compare L estimates in
hypothetical and real neurons with soma shunting having different L values.

Hypothetical cells were modeled as a cylinder with a soma when the L of the
cylinder was 0.5, 1.0, or 1.5. Time constants were estimated from computed
transients with the program DISCRETE. When L was 0.5 and some soma shunting
was large, the formula L0/τ1 overestimated L by 2-3 fold, but when L was 1.5, the
overestimate was only 10-15% regardless of the size of the shunt.

L can be estimated graphically if τ0 and τ1 in a neuron with an extensive dendritic tree differ by a factor of four, and the only factor causing this difference is soma shunting, then is approximately 0.5.

L0/τ1 estimates were computed for models of a motoneuron having different dendritic Rm and degrees of shunting. Estimates of τ1 in these motoneuron models were complicated by the fact that no time constant after τ0 had a coefficient larger than 2% of the signal. Overestimates of L by L0/τ1 were larger when dendritic Rm was large than when dendritic Rm was small, but the magnitude of the overestimates depended on the size of the shunt and on whether 2, 3, or 4 time constants could be resolved from the data.

In conclusion, L0/τ1 estimates computed for cells with soma shunting have overestimated L by a factor of 2 in electrotonically short cells but by only 0-20% in electrotonically long cells.

605.5
COMPUTER MODELS OF DENDRITIC EXCITABILITY. I. SEGEV* and
V. Rall. Dept. Neurobiol. Hebrew Univ. Jerusalem, and

Computer models with excitable membrane at different
dendritic locations were used to gain insights into the
possible functional roles of dendritic excitability.

Simulations were on theoretical neurons and on a reconstruc­
ted Purkinje cell from guinea-pig cerebellum. A previous report (Segev and Rall, Neurosci. Abstr. 12:726) focused on the "chain reaction" of action potential firing be­
tween clusters of excitable spines at distal dendritic
branches. Here we modeled trees with many "hot spots" (on
dendritic shafts and on spines). The main findings are:

a) The chain reaction tends to spread distally; it fails to­
ward the soma.

b) The propagation delay of the chain reaction
may be significant (tens of msec), depending on the
passive membrane time constant and on the kinetics of the
excitable branches.

c) Timing between different excitatory inputs distributed along the tree is critical for the de­
gree of firing within the tree; when inputs to proximal
regions are delayed (to allow for depolarization from dis­
tal inputs to reach the proximal sites), a larger part of the
neuron is more likely to fire than when all inputs are
simultaneous.

d) Inhibition is very effective in restrict­
ing the chain reaction to only parts of the tree. Strong
somatomotor inhibition tends to block the spread of the chain
reactions anywhere in the tree. The possible functional role
for cellular plasticity will be discussed.

605.6
ELECTROTROTONIC TRANSFORMS OF HIPPOCAMPAL NEURONS. A.M. Zador, B.J. Claiborne,* and T.H. Brown. Dept. of Psychology, Yale University, New Haven, CT 06520 and Div. of Life Sciences, Univ. of Texas, San Antonio, TX 78285.

The electrototonic transforms of dendrites is of central importance to their potential for information processing. This structure has traditionally been analysed using concepts like electronic distance and length, which have only limited applicability to cells with complex morphology. We present a new graphical method that provides direct insight into key aspects of a neuron's electrototonic structure. The method is based on the attenuation of voltage from location i to following current injection at i. Specifically, the length of each segment in an anatomically recon­structed neuron is replaced by the log-attenuation Lij = −log(Aij) across the segment, as calculated using a compartmental simulator. The log-attenuation is a generalization of the concept of electrototonic distance to morphologically complex trees, and is identical to X in the case of an infinite or semi-infinite cylinder. Because the log attenuation depends on the fre­quency and state of the input, many complemen­tary views of a neuron emerge. We propose this method to the attenuation of cur­rent and to temporal consequences of electrototonic transfor­mation.

This method is illustrated at the right. The neuron on the left is a 2734-compartment 3­
dimensional reconstruction of a region CA1 pyramidal neuron. On its right is a representa­tion of voltage attenuation from the soma with respect to a 100 Hz sinusoidal current. (Sup­ported by ONR and DARPA.)

605.7
MAGNETIC CHARACTERIZATION OF ACTION CURRENTS AT THE
PRIMARY BRANCH OF THE SQUID GIANT AXON. B.N. Friedman,* M.J. van
Faren,* and J.P. Wilkes. Jr. Department of Physics and Astronomy, Vanderbuilt
University, Nashville, TN 37235.

Magnetic field recording provides a more direct means of measuring the action
currents (ACs) in squid (Loligo pealei) giant axons. A room-temperature
magnetoelectric current lead system enables such studies by scanning a tunneling probe along the axon. This affords acquisition of nearly continuous AC profiles. We used this system to examine the currents as the action
signal approached and crossed the primary branch of the neuron. The figures show the net magnitude of stimulation a 2 cm axon (2 cm long) 3 mm distal to the ligated and cut proximal branch after 57 ±10 minutes (A) and (Andersen, Storm & Wheal, 1987, J.Physiol. 383 pp.509-526). The local attenuation of voltage from location i to following cur­rent injection at i. Specifically, the length of each segment in an anatomically recon­structed neuron is replaced by the log-attenuation Lij = −log(Aij) across the segment, as calculated using a compartmental simulator. The log-attenuation is a generalization of the concept of electrototonic distance to morphologically complex structures, and is identical to X in the case of an infinite or semi-infinite cylinder. Because the log attenuation depends on the fre­quency and state of the input, many complemen­tary views of a neuron emerge. We propose this method to the attenuation of cur­rent and to temporal consequences of electrototonic transfor­mation.

The method is illustrated at the right. The neuron on the left is a 2734-compartment 3­
dimensional reconstruction of a region CA1 pyramidal neuron. On its right is a representa­tion of voltage attenuation from the soma with respect to a 100 Hz sinusoidal current. (Sup­ported by ONR and DARPA.)

ACs in the two most proximal

electrotonic transforms of the

605.8
EFFECT OF QX314 ON SOMATIC AND DENDRITIC ACTION
POTENTIALS IN HIPPOCAMPAL CA1 CELLS. S.R. Collingt,* E.W. Stock LEY,* and H.Y. WEHAI.
Department of Physiology and Pharmacology, Southampton University,
Southampton, S09 3TU, UK.

Hippocampal CA1 pyramidal cells in vitro can be excited via stimulation of accessory fibres to the proximal and distal apical dendrites (Andersen, Storm & Wheal, 1987, J.Physiol. 383 pp.509-526). The local anaesthetic QX314 (35 mM) was injected intracellularly, and its effects on the proximally and distally evoked synaptic responses were monitored over time. The proximally evoked action potential (AP) was totally blocked after 57±10 minutes (A) and the distally evoked AP was present up to 88±14 minutes (B). The extracellular synaptic potentials were still present after this time. In control recordings there appears to be a pre-AP component which contributes to the AP peak. Here the threshold event. This component also appears to be sensitive to QX314.

The data suggests that there is more than one triggering site for APs in pyramidal cells and that these sites are spatially separate. The shapes of the synaptic potentials as well as their generation from these sites can be explained by the electrical properties of these cells. A compartmental cable model was used to confirm that APs evoked by a dendritic spike which appears as a prepotential at the soma.

Supported by MRC and Wellcome Trust grants. The QX314 was donated by Astra Pain Control, Solna, Sweden.

605.3
EFFECT OF NH₄Cl ON EXCITATORY TRANSMISSION IN CAI PYRAMIDAL NEURONS RECORDED INTRACELLULARLY. H. Pachaus,* P. Segert, and J.C. Zilber. Dept. Physiol. & Biophys. Dalhousie U.Halifax,N.S. Canada B3H 4H7

We have shown that NH₄⁺ blocks synaptic transmission from Schaffer collaterals to CA1 neurons without inhibiting the evoked release of glutamate but suggesting a postsynaptic block. However NH₄⁺ was only a weak inhibitor of firing induced by electrophysiological stimuli. To clear up this discrepancy, we applied 10-20 μM quisqualate (QUIS) or NMDA to slices, while the membrane current was recorded with single-electrode voltage clamp. 2-4 μM NH₄Cl induced an inward current and had opposite effects on QUIS and NMDA-induced currents: it abolished the QUIS current and greatly potentiated that of NMDA. Since transmission involves mostly QUIS receptors, block of the QUIS current explains the inhibitory effect of NH₄⁺ on transmission. The action of NH₄⁺ is probably due to depolarization of inadequately clamped distal dendrites, because the potentiation of the NMDA effect is not seen without Mg²⁺.

(Supported by MRC, P.F. is an MRC Fellow).

The polyribosomal aggregates observed within or at the bases of dendritic spines have suggested that some proteins, produced quickly and localized in such groups, might contribute to synaptic plasticity. Protein synthesis has been demonstrated in synaptosomal preparations by several groups. We have added a depolarizing dose (40 mM) of K+- induced depolarizations, preparations, on ice in the presence of cycloheximide. By measuring the fraction of total RNA trapped in polyribosomes at intervals (1.5, 5, 10, 30 min) we have been able to show that some pre-formed protein rapidly associates with mRNA; this was verified by centrifugation on a continuous sucrose density gradient. Ribosomal loading peaks at 5-10 min and then declines. Incorporation of labelled methionine into TCA-precipitable polyribosomes is transiently accelerated.

Calcium chelators reduce the steady-state size of polyribosomal complexes, but do not eliminate the response to depolarization, suggesting that there is mobilization of intracellular Ca++ stores. We suggest that depolarization-related changes may affect binding and release of translation initiation complexes with messenger RNA found near synaptic junctions. Such rapid, local protein synthesis may have implications for synaptic efficacy changes.

Sustained by DNR 0014-89-1556 and MH35311.


Previous studies over the range of 37°C to 27°C suggested that different channels have different temperature sensitivities (e.g., Thompson et al., J. Neurosci., 5:817, 1985). The intent of this study was to extend consideration of thermal effects to a lower range of temperatures. Conventional intracellular recording and current injection techniques were used to elicit spike trains in CA1 pyramidal cells at 20°C to 25°C. We measured the amplitude and duration of the action potential following a slow afterhyperpolarisation (AHP). The normalized duration of the AHP (measured at 50% of peak height) was 750 ± 30 msec/mV. The duration of the AHP is greatly extended at 25°C compared with measurements at 39°C, an increase of approximately 75%. In contrast, the width of simple action potentials did not increase as much as the AHP. Data indicate that different ionic currents can have markedly different thermal sensitivities and that the sensitivity of the Ca2+-activated potassium current responsible for the AHP may reflect greatly altered calcium metabolism at low temperatures. Supported by NSF Grant BNS-88-19973.
THAPSIGARGIN STIMULATES CALCIUM INFLUX IN NEURONS.

Using the neuronal tumor cell line SK-N-SH, we demonstrate that receptor-mediated activation of phosphoinositide hydrolysis not only causes the release of Ca2+ from intracellular stores but also causes a concomitant influx of Ca2+ from the extracellular space. Treatment of fura-2 loaded cells with 1μM thapsigargin (TG) in the presence of extracellular Ca2+ causes a depletion of the inositol 1,4,5-trisphosphate-sensitive intracellular Ca2+ store (ICa+o) and a sustained elevation of the extracellular Ca2+ concentration (ICa++e). TG produces only a transient increase in [Ca++\textit{i}]. These data suggest that TG activates both the release of intracellular Ca2+ and the influx of extracellular Ca2+. This supports the proposal that the depletion of the inositol 1,4,5-trisphosphate-sensitive intracellular Ca2+ pool initiates extracellular Ca2+ influx.

PHARMACOLOGY OF INOSITOL 1,4,5-TRISPHOSPHATE (IP3)
SENSITIVE CALCIUM INFLUX (ISCI) IN XENOPUS OOCYTES.

Xenopus oocytes injected with IP3 develop an increase in the plasmalemma permeability to calcium ions. Increases in [Ca+]o were assayed via activation of the oocyte’s native Ca-sensitive Cl channel. When oocytes were loaded with >5pmol IP3 and kept in Ca-free solution, the increase in Ca membrane permeability lasted for more than 1h. We studied the pharmacology of this response in order to explore the mechanism involved in ISCI. The ISCI was not sensitive to organic voltage-sensitive calcium channels (VSCC) modulators (Bay K, nifedipine, verapamil, MK 801) and exhibited low sensitivity to inorganic VSCC blockers (Cd, Ni, La). ISCI was not affected by agents that modulate Na/Ca cotransport (amiloride, Na/Li substitution, Na/NMG substitution). Lowering the extracellular pH to 6.5 or applying the protophosphate (FCCP) completely blocked the ISCI. Application of the Ca-ATPase blocker vanadate did not affect this current. However, addition of the H-ATPase blocker (NBD-Cl) potentiated this current and at high concentrations enhanced Ca entry by itself. The pharmacology may suggest that H/Ca exchange (possibly as part of the H-ATPase complex) contributes to ISCI.

GADOLINIUM SELECTIVELY BLOCKS Ca++ INFLUX THROUGH VOLTAGE-DEPENDENT CALCIUM CHANNELS IN CEREBRAL VENOUS ENDS.

The pharmacological modulation of Ca++ entrance is a matter of debate and there is a lack of suitable agents which potently and selectively block Voltage-Dependent Ca++ Channels. Ca++ influx in Percoll-purified rat brain synaptosomes was investigated either through the measurement of optoelectrical Ca++ levels, monitored by Fura-2 or by the evaluation of the fast phase of 55mM K+-elicited 45Ca++ influx. The trivalent rare earth lanthanide, Gd+++ (1-30 μM) also inhibited the fast phase of 45Ca++ influx induced by 55mM K+. Among the organic Ca++ entry blockers, dihydropiridines were completely ineffective, whereas the phenylalkylamine Verapamil (100 and 1000μM) and the aminoglycoside Neomycin (300 and 1000μM) prevented [Ca++\textit{i}] elevation and 45Ca++ fast phase influx only at elevated concentrations. These results suggest that Gd+++ is a suitable tool to selectively block Ca++ influx in cerebral nerve endings. (Supported by C.N.R. and M.U.R.S.T. 884416 grants to L.A.)

606.6 EVALUATION OF VOLTAGE- AND Ca++-DEPENDENT MECHANISMS IN THE INACTIVATION OF THE Ca++ CURRENT IN HELIX NEURONES.
I.M. Stanford* and J.E. Chad, Dept. of Physiology and Pharmacology, Univ. of Southampton, SO17 1TD, UK.

The inactivation kinetics of the whole cell Ca++ current measured in isolated Helix neurones has been widely interpreted as due to separate Ca++- and voltage-dependent processes, however the relative contributions of these processes are less widely agreed. We have attempted to separate the two processes by manipulation of the intracellular buffering agents (10mM) and extracellular divalents (50mM). Experiments were conducted on isolated dialysed, whole cell patch clamped neurones, using conditions designed to isolate the divalent current through Ca++- channels. Inactivation produced by a 50ms prepulse 100ms before a test pulse generated maximum inward current, and inactivation with single 200ms pulses to different membrane potentials were measured. In the prepulse experiments, maximum inactivation was obtained with Ca++ as charge carrier and no added buffering (values given as mean ±SD, n>5; 0.23 ±0.06), reduced inactivation was obtained with Ba++ and EGTA (0.1 ±0.03), and Ba++/BAPTA further reduced inactivation. Even under these conditions, the inactivation was not monoexponential with regard to prepulse voltage. The inactivations of currents during single 200ms test pulses with a series of BAPTA analogues, showing a dependence related to the Ca++ K of the buffer. Dimethyl-BAPTA (Kb 400mM) permitted less inactivation (ICa++ 0.32 ±0.03; Ib++ 0.18 ±0.04) than was observed with 4,4’ difluoro-BAPTA (Kb 2450mM; ICa++ 0.57 ±0.09; Ib++ 0.30 ±0.07), for both Ca++ and Ba++. Thus either Ba++ can produce some divalent dependent inactivation itself, or acts via an elevation in intracellular Ca++, due to contamination or displacement from buffers. Ca++ buffering conditions reflect voltage-dependent inactivation alone.

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606.8 ZINC (Zn++) BLOCKS VOLTAGE GATED CALCIUM CHANNELS IN CULTURED RAT DORSAL ROOT GANGLION CELLS. D. Büsselberg, M.L. Evans, D.O. Carpenter, and H.L. Haas.
Institute für Physiologie der Heinrich-Heine Universität Düsseldorf, D-4000 Düsseldorf, FRG, SmithKline Beecham, Harlow, Essex CM19 5AD, UK, and NYS Dept. of Health and School of Public Health, Albany, NY 12201-0590, USA.

Dorsal root ganglion cells (DRGs) exhibit three types of voltage-dependent calcium channels. We have cultured DRGs from 2 to 4 day old rat pups and obtained whole cell patch clamp recordings of calcium channel currents. THEY TO 5 days culture. Transient currents, carried by barium cations, were recorded in the following external solutions: TEA Cl 135 mM, HEPES 10 mM, glucose 10 mM, BaCl2 10 mM, MgCl2 1 mM, TTX 0.002 mM. A calcium based internal solution containing 2 mM ATP was used in the recording pipette. The divalent cation Zn++ (1-1000μM) dose-dependently counteracted the increase of Ca++ influx induced by 35mM K+ . 0.09 mM (1-100 μM) also inhibited the fast phase of 45Ca++ influx induced by 55mM K+. Among the organic Ca++ entry blockers, dihydropiridines were completely ineffective, whereas the phenylalkylamine Verapamil (100 and 1000μM) and the aminoglycoside Neomycin (300 and 1000μM) prevented [Ca++\textit{i}] elevation and 45Ca++ fast phase influx only at elevated concentrations. These results suggest that Gd+++ is a suitable tool to selectively block Ca++ influx in cerebral nerve endings. (Supported by C.N.R. and M.U.R.S.T. 884416 grants to L.A.)
DIVALNT IONS FROM STAINLESS STEEL HYDOPERMIC NEEDLES REDUCE NEURONAL CALCUM CURRENTS.
S.C. Nam and P.E. Hockberger. Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.
Stainless steel hydoperoxide needles are widely used in both biomedical research and clinical practice because they are cytotoxic and corrosion-resistant. The most common type of stainless steel (304) is formed primarily with chromium and nickel (Ni), although other chemicals may be present depending upon the manufacturer’s raw materials and processing steps. We have compared nickel- and chromium-plated stainless steel needles from several manufacturers on their ability to affect voltage-dependent Ca2+ currents in isolated chick (E13-E16) DRG neurons and rat (P7-P13) cerebellar Purkinje cells. Our protocol involved allowing external saline (in mM: choline-Cl, 120; CaCl2, 3; MgCl2, 2; HEPES, 10, pH 7.3) to sit in needles for various durations of time (20-180 min) and then analyzing Ca currents during local application of the test salines. Salines exposed to needles with brass hubs, but not those with plastic hubs, for 20-30% reduced the release. Moreover, exposure resulted in proportionately greater reductions. These reductions were recorded immediately upon saline application and were completely reversible. This early analysis of the test salines using JCP-AE spectrophotometry revealed that Cu and Zn (but not Ca, Cr, Fe, Pb, Mo, or Ni) were released from brass needles. The amount of Cu2+ measured in Ca2+ released Cu2+ in both cell types by 65% (n=6), whereas 100mM ZnSO4 reduced the release by 20-50% (n=6). These results indicate that Cu (and possibly Zn) ions released from stainless steel needles during exposure are not responsible for the Cu2+ release.

This research was supported by NIH grants #NS-26915 and #NS-17489. ICP-AE spectroscopy was performed by Samia Sfaiha, Chemistry Dept., Northwestern University, Evanston, IL 60201.

LEAD IONS (Pb2+) PERMEATE THROUGH CALCIUM CHANNELS. K.L. Tompasi and J.B. Susskind. Dept. of Physiol., and Biophys. Univ. of Cincinnati Sch. of Med. Cincinnati, OH 45267.
Neurotoxic properties of lead are associated with the entry and intracellular interaction of Pb ions with Ca2+-mediated cell functions. In this study we have employed Fura-2 to monitor Pb2+ entry into isolated, bovine chromaffin cells exposed to low (micromolar) concentrations of Pb. Similar to lead, high-K+ environments, the accumulation of Pb2+ in chromaffin cells was a linear function of Pb2+ influx, indicating passive leakage. Exposure of cells to high K+ stimulated the entry of Pb2+ into the cell in a function of Pb2+ influx. The Pb2+-depressed Pb2+ influx was further enhanced by calcium channel agonist bay K 8644 and blocked by channel antagonist nifedipine, suggesting the involvement of L-type calcium channel. The Pb2+ influx in Pb2+-entrapped cells was a linear function of Pb2+ concentration at Pb2+ concentrations .1 and Pb2+ concentrations .3.
The possibility of Pb2+ entry and Pb2+ release was studied in sympathetic cultures of rat isolated sympathetic neurons (SN), where Pb2+ entries were 50-150 fold greater than that of Pb2+ influx at Pb2+ concentrations .1 and Pb2+ concentrations .3.

In conclusion, the Pb2+ influx and Pb2+ release were 50-150 fold greater than that of Pb2+ influx at Pb2+ concentrations .1 and Pb2+ concentrations .3.

Supenmized Taces from 2 individual taqments, (a) 60% depletions of 4ms duration in 10mM Ca2+ (a). 32% depletions of 76ms duration in 10mM Ca2+ (b).

An equal number of stimulus pulses applied at increasing frequency is known to increase release of norepinephrine (NE) in neuroeffector organs. Kirpekar et al. (Raunyn-Schadel's Arch. Pharmac. 287:205, 1975) proposed that greater influx of Ca2+ may occur at high frequency. This idea was tested by measuring cytosolic Ca2+ concentration ([Ca2+]i) in growth cones in cultured sympathetic neurons (SN). Release and reuptake of NE were monitored using jcd-1-2, 3.5x10-8 to 3.5x10-7 M when 150 pulses were delivered at 1 Hz and at 10 Hz, respectively. The net increase (stimulated - basal) in [Ca2+]i at 1 Hz was 210 nM and at 10 Hz 310 nM. The rate of rise in [Ca2+]i at 10 Hz was much faster than at 1 Hz. Continuous stimulation of 1 Hz at 1 Hz for 3 min increased release in [Ca2+]i and [NE](t).

At 10 Hz there was a greater and stronger sustained rise in [Ca2+]i and [NE](t). 55 nM Ca2+ caused a sharp rise in [Ca2+]i, and [NE](t) release but both parameters rapidly declined during 3 min exposure to K+. We conclude that net increase and rate of increase of [Ca2+]i determine [NE](t) release at different frequencies. We also show that sustained depolarization (excess K+) inactivates Ca2+ entry and [NE](t) release while high frequency stimulation maintains the release.

EFFECTS OF TEMPERATURE ON CALCIUM CURRENT OF BULLFROG SYMPATHETIC NEURONS. F. van Lunteren, K.S. Limjil and S.W. Jones. Departments of Medicine, Physiology and Biophysics, and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.
The present study assessed the effects of temperature on the magnitude and kinetics of voltage-activated calcium current in dissociated frog sympathetic neurons. Calcium current increased with increasing temperature, peak current being 1.5 to 2 fold greater at 30°C compared to 20°C. Estimates of the magnitude of the effects of temperature on peak current amplitude were complicated by the considerably faster run-down of current at high compared to low temperatures. Activation and deactivation kinetics became significantly faster over time during current run-down, but were substantially faster at high compared to low temperatures. The time constant of activation (over a range of -30 to -20 mV) was 4 to 7 times larger at 20°C compared to 30°C (eg. 4.18 ± 0.75 vs. 0.61 ± 0.10 msec at -1 0mV; mean ± SD; n=6). Further, the rate of inactivation became faster over time and with increasing temperature. These data suggest that calcium currents in DRG cells are different from dihydropyridine-sensitive cell types. This research was supported by NIH grants #NS-26915 & #NS-17489.
606.17

EFFECTS OF ISOFLURANE ON CALCIUM CURRENTS RECORDED BY THE WHOLE-CELL PATCH CLAMP TECHNIQUE IN NEOCORTICAL SLICES. B. Hutcheon, E. Flei and P.D. Reiner. Dept. of Pharmacology and Therapeutics, Dept. of Anaesthesia, and Dept. of Psychiatry, Univ. of British Columbia, Vancouver, Canada. V6T 1Z3.

Rat sensory-motor cortical neurons were voltage-clamped using whole-cell patch clamping techniques in an in vitro slice preparation. Calcium currents were isolated by the extracellular application of 4-aminopyridine, tetraethylammonium, cesium, and tetraodoxin. Patch pipettes contained 110 mM NaCl and 80 mM CaCl2 in the pipette was buffered to 10 nM. High-threshold (-30 to -10 mV) inward currents were evoked by depolarizing pulses from holding potentials near -80 and -40 mV. The evoked Ca2+ currents were completely blocked by 3 mM isoflurane applied in the bathing solution. Lower concentrations of isoflurane reduced the peak amplitude, slowed the onset, and raised the threshold of activation of the Ca2+ currents. The depressant effects were seen after 6-12 minutes of isoflurane application (flow rate >2 ml/min), and recovered within 1-2 minutes. The present studies reveal that isoflurane may depress neuronal excitability by blocking a high-voltage activated Ca2+ channel.

606.18

CHRONIC DEPOLARIZATION ELEVATES INTRACELLULAR CALCIUM LEVELS IN CULTURED RAT MYENTERIC NEURONS. D.J. Fickbohm1 and A.L. Willard1,2, Currie, in Neurobiology1 and Dept. of Medicine2, University of North Carolina, Chapel Hill, NC 27599-7545.

We have been studying the effects of chronic depolarization on survival and development of rat myenteric neurons in cell culture. Previously, we found that in medium containing elevated potassium (25 mM), which depolarizes these neurons to a mean resting membrane potential of -40 mV, alters their development in several ways: it enhances survival, it increases neuronal size, and it suppresses voltage-dependent Ca currents. Here, we report that chronic depolarization causes persistent elevation of intracellular Ca (Ca2+) and that dipyridophosphinic (DPP) antagonists prevent this effect.

Myenteric neurons dissociated from intestines of 2-3 d old rats pups were grown in cell culture for 3-6 d in medium containing control (3 mM) or elevated (25 mM) KCl. [Ca2+]i was estimated with Indo-1 and fura-2. Although the 2 dyes gave different estimates of [Ca2+]i, both methods revealed significantly higher [Ca2+]i in depolarized neurons. The DHPs nitrendipine and nifedipine (5 μM) prevented or reduced the effects of elevated potassium. The estimated values of [Ca2+]i, dependent critically on the method of calibration. “In situ” calibration (using isoflumates) yielded values roughly half those estimated with “in vitro” calibrations, possibly due to vigorous intracellular Ca buffering mechanisms reducing the apparent Rmax. With in vitro calibration, [Ca2+]i was 37 ± 9 nM (Indo) or 187 ± 12 nM (fura) for depolarized neurons and 196 ± 5 nM (Indo) or 126 ± 9 nM (fura) for control neurons (all n’s > 100).

These results support the hypothesis that persistent elevation of [Ca2+]i is a mechanism by which chronic depolarization alters the development of myenteric neurons in culture. Supported by NIH grants to A.LW.

606.19


Our working hypothesis for nodal function incorporates the idea that the axonal cell ensemble serves as a node in the physiology of action potential propagation. This study was designed to expand on previous observations indicating active participation of the Schwann cell in the functioning of the node of Ranvier. In order to test the potential role of Ca in nodal activation we developed a system for in vitro studies that combines simultaneous optical and electrophysiological recording of single fibers. Frog sciatic nerve was dissected and loaded with the membrane permeable Ca indicator fluo3-AM. Action potentials of single fibers were recorded with a fine suction microelectrode. These active fibers were followed to observe healthy looking active nodes of Ranvier after they entered a bundle of surrounding fibers. The Ca transients in the ensheathing Schwann cell were then recorded using a laser-scanning confocal microscope system. The flux ofCa2+ was monitored in the Schwann cell and did not stain the axon. This is probably due to physical barriers associated with the structure of the node of Ranvier in the PNS. Upon stimulation, an increase in intracellular [Ca2+]i concentration was observed in the paranodal region and other cytoplasmic pockets of the ensheathing Schwann cells. Ca transients were observed in the single fibers monitored with the microelectrode as well as in Schwann cells of surrounding fibers. These other fibers were presumably also ensheathing electrically active nerve fibers.

Fibers were also observed after the stimulation was halted. (The Ca2+ continued to increase briefly after the cessation of long and rapid stimulation. After a recovery to near prestimulation levels, repeated stimulation of shorter duration produced a fast increase in [Ca2+], which decreased more rapidly after cessation stimulation. This observation is interpreted to indicate that the Schwann cell response was not damaged by the initial long periods of stimulation. The physiological basis for these Ca transients will be investigated further.

606.20


“Ca2+” flux into 100μM pions of adult rat neocortex was measured at 25°C in HEPES/Hepes buffered HBSS (137mM Na+, 3mM K+, 1.25mM Ca2+) and also under conditions where K+ was substituted for Na+ up to 1000mM K+. After 30s incubation, elevated K+ induced a 3-fold increase in Ca2+ flux over basal conditions. At 45°C, half the flux was half maximal at 25mM, and reached a plateau at 50mM K+. The time course for 30mM K+-stimulated flux showed a rapid rise in Ca2+ from 1-30s followed by a plateau for 30-120s. A 60s pre-depolarization in 30mM K+ buffer containing zero Ca2+ did not affect the time course for Ca2+ flux, suggesting that the relevant Ca2+ channels are of the non-inactivating type.

K“(30mM)-induced Ca2+ flux was unaffected by TTX1μM, the NMDA antagonists CPP(100μM) and MK-801(1μM), and the non-NMDA antagonist NBQX(100μM). With regards to the Ca2+ channels, agonists of the NMDA receptor of the NMDA type and the non-NMDA type were inactive in their own right. In contrast, Ca2+ flux was reduced by a potent potassium concentration of Ca2+ > Ni2+ > Co2+. The following rank potency order, with IC50 ranging from 250-500μM, was seen with various classes of Ca2+ channel antagonists: washout > nifedipine > dextrorphan > dipropylamine > neomycin. n- Conotoxin GVIA(1μM) was inactive. These relatively high IC50 values and the inactivity of n-conotoxin GVIA(1μM) are consistent with well-classified Ca2+ channels pharmacologically, suggesting the possibility of a novel Ca2+ channel subtype.

The voltage-dependent anion channel (VDAC) is a 32 kDa protein that has been localized to the outer membrane of mitochondria. VDAC has been shown by other investigators to function as: 1) an anion channel, 2) a non-specific pore through which many solutes may pass to gain access to the mitochondrial inner membrane, and 3) an intracellular receptor for cytokines, such as TNFα. Rat brain VDAC has been purified by conventional procedures and this protein cross-reacts with an anti-kidney VDAC antiserum. Immunohistochemically, VDAC is shown to be very heterogeneous. While the localization is primarily to glia, specific neurons were positively identified with the anti-VDAC antiserum. The glomerular layer of olfactory bulb, thalimus, caudate putamen, supraoptic nucleus, pontine nuclei, CA4 region of the hippocampus (glia and pyramidal cells), deep cerebellar nuclei, cell bodies and dendrites of Purkinje cells were the regions most intensely stained. These results also were the most intensely stained with histochemical stains for mitochondrial enzymes: cytochrome oxidase, monoamine oxidase, and succinate dehydrogenase.

VDAC localization is thus coincident with the regions of greatest mitochondrial density in rat brain. These results demonstrate heterogeneous mitochondrial populations in glia and neurons.

607.2 SECRETORY VESICLE-SPECIFIC CHANNELS FROM THE NEUROHYPOPHYSIS. K.E. Krebs, S.H. Goldberg* and G. Ehrenstein*. Lab. of Biophysics, NINDS, National Institutes of Health, Bethesda, MD 20892.

Two distinct populations of secretory vesicle are present in nerve terminals in the bovine neurohypophysis: 100-300 nm diameter neurosecretory granules (NSG) that contain peptide neuropeptides, and 40-60 nm microvesicles (MV) of unknown function that are biochemically related to small synaptic vesicles of presynaptic nerve terminals. We now report that these two classes of neurohypophyseal vesicle contain distinct ion permeable channels. NSG and MV from bovine neurohypophysis were prepared by differential centrifugation, followed by separation on a 3000 A controlled pore glass bead column, to yield highly purified vesicles on the basis of size. Purified NSG or MV were added to the cis side of painted lipid bilayers (80% PE/20% PC). Fusion of NSG with the bilayer resulted in the incorporation of a calcium-dependent (peak open probability of 80% at 300 nM Ca2+), impermeable selective chloride-permeable channel (>150 pS) that was blocked with 400 μM DIDS. In contrast, the fusion of MV with the bilayer resulted in the incorporation of a potassium-permeable channel (>100 pS) that appeared to be similar to channels identified in small synaptic vesicles purified from presynaptic nerve terminals of bovine brain.


I have used the whole-cell (w) patch clamp technique to simultaneously monitor changes membrane capacitance (Cm) and activation of CL- conductance (gc) in the colonic cell line, T84. An EPC-9 amplifier was used to assess a possible relationship between vesicle fusion events stimulated by [CaMP]I and CL- conductance. Cm and gc were determined by analyzing the RC decay of the membrane current in response to a voltage pulse. When recording in (mM) NaCl 155, MgCl2 2, CaCl2 2, Hepes 10 (bath) and Cs glutamate 130, MgCl2 2, Hepes 10, ATP 2, GTP 0.2 (with [CaMP]I buffered to <10-6 M with EGTA (pipet)), <────> cells showed an increase in Cm, while gc was activated in 9/12 cells. In contrast, when 100 μM [CaMP]I was included in the pipet solution, Cm increased in 5/8 cells and gc was activated in 7/8 cells. Increases in Cm ranged from 0.5-4.0 pF, or ~10-20% of cell membrane area. No cells (n=6, 100 μM [CaMP]I) showed a change in Cm when 50-100 μM DIDS was added to the bath to block CL- conductance.

Recordings made in Na glutamate (bath) and K+ or Cs glutamate 100 mM [CaMP]I (internal) also prevented increases in Cm (12/13 cells). These observations suggest that activation of gc is insufficient to stimulate an increase in Cm, but is required for [CaMP]-dependent fusion of vesicles to the plasma membrane.

This work was supported by the CFF and DK 38518. I thank Dr. R.A. Prinzell for encouragement and support.

607.5 EFFECTS OF MONOSIALOGANGLIOSIDE GM1 ON RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS. P. Miu and K. Krnjević. Anesthesia Research Dept., McGill University, Montréal, Quebec, Canada, H3G 1Y6.

When recorded with Cs+ filled electrodes, and superfused with ACSF at (33°C) containing TTX (1 μM), TEA (10 mM), Cs (4 mM) and 4-AP (100 μM), pyramidal cells consistently responded to exogenous monosialoganglioside GM1 with an increase in the leak conductance (gL) and an inward shift in the holding current (Ih) (Agopyan et al., 1990, Soc. Neurosci. Abstr., 16:623).

Under identical conditions (Vh = -40 mV) but recording with Cs+ acetate-filled electrodes, 4 out of 6 CA1 pyramidal cells responded to 1 μM GM1 application with a reduction in gL by 7.9 ± 2.1% (n = 5; p < 0.025, 1 tail t-test), and a slight outward shift in Ih of 0.007 ± 0.007 nA; n = 7; N.S., 2 tail t-test). As observed previously, 1 μM GM1 reduced the high threshold calcium currents by 39.3 ± 6.5% (n = 5; p < 0.003, 1 tail t-test). However, the GM1-induced reduction in the high threshold calcium currents could be reversed by 1 mM kynurenic acid.

In conclusion, the present findings suggest that a) GM1 may activate chloride conductance and b) GM1 facilitates TTX-insensitive glutamate release as shown by the enhanced excitatory post-synaptic currents (Miu and Krnjević, 1990, Curr. 21:261). The potentiated glutamate release would increase the intraneuronal Ca2+ concentration, and thereby trigger Ca2+-dependent Ca2+ inactivation (Ecker and Chud, 1984, Prog. Biophys. Molec. Biol., 44:215), thus explaining the reduction in high-threshold calcium currents.

This project was supported by MRC of Canada, and by Bydfia.

607.6 SECRETORY VESICLE-SPECIFIC CHANNELS FROM THE NEUROHYPOPHYSIS. K.E. Krebs, S.H. Goldberg* and G. Ehrenstein*. Lab. of Biophysics, NINDS, National Institutes of Health, Bethesda, MD 20892.

Two distinct populations of secretory vesicle are present in nerve terminals in the bovine neurohypophysis: 100-300 nm diameter neurosecretory granules (NSG) that contain peptide neuropeptides, and 40-60 nm microvesicles (MV) of unknown function that are biochemically related to small synaptic vesicles of presynaptic nerve terminals. We now report that these two classes of neurohypophyseal vesicle contain distinct ion permeable channels. NSG and MV from bovine neurohypophysis were prepared by differential centrifugation, followed by separation on a 3000 A controlled pore glass bead column, to yield highly purified vesicles on the basis of size. Purified NSG or MV were added to the cis side of painted lipid bilayers (80% PE/20% PC). Fusion of NSG with the bilayer resulted in the incorporation of a calcium-dependent (peak open probability of 80% at 300 nM Ca2+), impermeable selective chloride-permeable channel (>150 pS) that was blocked with 400 μM DIDS. In contrast, the fusion of MV with the bilayer resulted in the incorporation of a potassium-permeable channel (>100 pS) that appeared to be similar to channels identified in small synaptic vesicles purified from presynaptic nerve terminals of bovine brain.
TETRAETHYLMAMMONIUM (TEA) AND RELATED QUATERNARY AMMONIUM IONS BLOCK CHLORIDE CHANNELS IN CEREBRAL CORTEXAL NEURONS.
D.V. Sanchez and A.R. Betz. Physiology Department, UT Southwestern Medical Center, Dallas, TX 75235-9040
TEA and other symmetric quaternary ammonium ions (QAs) are commonly used to block chloride channels. We have found that QAs also block current through fast chloride channels in rat cerebral cortex. Symmetric QAs with alkyl chain lengths of 1-6 carbons were studied using excised, inside-out membrane patches with either an extracellular or internal blocker. Unlike TEA, which reduced single channel conductance, longer chain QAs reduced mean open duration with no effect on conductance. QAs blocked current through these channels in a voltage-dependent manner when applied externally. Internal blocking ions were dependent on voltage. The figure shows mean open duration with 0.3 mM external blocker (currents filtered at 3kHz, -3dB) and demonstrates that, similar to internal QA block of K channels, the blocking ions have a higher affinity for the channel (lower Kp).
Increased concentration of blocker decreased open duration suggesting that the blocker binds the channel's open state. Supported by NIH grants HL07160 & GM39731.

EXPRESSION OF GLYCINE RECEPTOR SUBUNITs IN HIGHER BRAIN REGIONS. I. Kuklin*, A. Karyatov*, Y. Maule*, M. L. Malicio*, H. Betz. Max-Planck-Institut für Hirnforschung, Deutscherstrasse 46, D-6000 Frankfurt 1, Germany
The postynaptic glycine receptor (GlyR) is a ligand-gated chloride channel which mediates inhibition of neuronal activity in the vertebrate central nervous system. Two different subunits of 48 kD (a) and 58 kD (β) form a pentameric receptor. Immunological and molecular cloning data have disclosed heterogeneity of GlyR α subunits during development. In the CNS of rat, α1, α2, α2*, and α3 subunit genes are expressed.
We have used molecular cloning of cDNA and genomic sequences to demonstrate the expression of alternative splicing products of the α2 transcript (α2A and α2B). Furthermore, we have compared the expression of these mRNAs with that of other GlyR mRNAs in various regions of the rat CNS, using in situ hybridisation techniques. The α2A, α2B and α2* mRNAs are expressed in spinal cord and various brain regions and are down-regulated during development.
In contrast, α1 subunit mRNA is expressed throughout the CNS at very high levels, suggesting the existence of additional α1 subunit genes.

INCREASED CYTOSOLIC CHLORIDE CAUSES GLYCYNE-ACTIVATED CHLORIDE CHANNELS TO SHIFT TO LOWER PERMEABILITIES. A. M. Mühlen* and A. R. Martin. Dept. of Physiology, Univ. of Colorado Sch. of Med., Denver, CO 80220.
Outside-out membrane patches were pulled from mouse aorta and cells maintained in culture for 2-4 weeks. Single channel activity was produced by application of glycine to the outer surface of the patch at a concentration of 10 μM. The extracellular (bath) chloride concentration, [Cl]o, was maintained at 157 mM. With a cytoplasmic (electrode) concentration, [Cl]i, of 157 mM, the channel conductance was 1.5 nS, 155 pS, and 13 pS. A few openings at 33 and 47 pS were also observed. The equivalent permeabilities and relative number of occurrences of the major open states were 14 (41%), 10 (23%) and 7 (23%) x10^-14 cm^3/sec, respectively. With increased chloride in the electrode ([Cl]o = 20 mM), similar permeabilities were observed, but with different distributions: 14 (35%), 10 (54%) and 7 (23%) x10^-14 cm^3/sec. The remaining openings were to a still smaller permeability of 4 x 10^-14 cm^3/sec (10%). Thus it appears that one effect of increased [Cl]o is to shift the preferred channel openings to lower permeability states.
(Supported by N.I.H. Grant NS09660.)

DUAL EFFECT OF GABA ON NEUROSECRETORY CELLS OF CRAYFISH.
Symmetric QAs with alkyl chain lengths of 1-6 carbons were studied using excised, inside-out membrane patches with either an extracellular or internal blocker. Unlike TEA, which reduced single channel conductance, longer chain QAs reduced mean open duration with no effect on conductance. QAs blocked current through these channels in a voltage-dependent manner when applied externally. Internal blocking ions were dependent on voltage. The figure shows mean open duration with 0.3 mM external blocker (currents filtered at 3kHz, -3dB) and demonstrates that, similar to internal QA block of K channels, the blocking ions have a higher affinity for the channel (lower Kp).
Increased concentration of blocker decreased open duration suggesting that the blocker binds the channel's open state. Supported by NIH grants HL07160 & GM39731.

D-Tetrahydrocannabinol (THC) is a major active compound present in marijuana (Cannabis sativa). In human THC alters nausea, pain, anxiety, muscle tension, and memory. Many of these effects are similar to those produced by benzodiazepine barbiturates, and alcohol which act on the chloride channel regulated by the gamma aminobutyric acid (GABA) type A receptor. Here, we examined the effect of THC on the GABA chloride channel complex.
Synaptoneurosomes were prepared from rat whole brain and 36-chloride ion (36-Cl) influx was assayed, using a slightly modified version of a standard procedure (Harris, R.A. and Allen, A.M., Science 225, 1108, 1984). Basal Cl- influx was enhanced 84-195% (average 22%) when 0.1-1.0 μM THC was present in a colloidial suspension with 3%, w/v, polyvinylpyrrolidone-40.
When the effects of GABA on basal 36-Cl influx were examined, half maximal stimulation was found at 15 μM GABA with a maximum being reached by 100 μM at which point influx was at least doubled. In the presence of 1 μM THC, there was a leftward shift of GABA stimulation so that it was enhanced by at least 20% for both 3 and 10 μM GABA, falling off at 30 μM GABA, so that by the 100 μM maxi, no increase was produced. Generally similar results were observed with muscimol. These effects suggest THC released endogenous GABA.
100 μM Picrotoxin, or 100 μM of the GABA-A antagonist, bicuculla, reduced both GABA-muscimol and THC induced chloride increases to basal levels. 100 μM Baclofen or 100 μM 2-hydroxybococaine, GABA-B ligands, were ineffective, as was 10 μM caffeine.
THC appears not to compete directly with the chloride channel complex receptors. It is proposed that THC binds the newly identified cannabinoid receptor, whose distribution parallels that of the GABA-A receptor, to somehow mobilize endogenous GABA. This opens the chloride channel to produce THC behavior.
Palytoxin (PTX) is a potent venoconstrictor and cytotoxin isolated from marine invertebrates of the genus Palythoa. The precise mechanism of the toxin action is not clear but has been attributed to increased cation conductance in the plasma membrane of affected cells. We have investigated the actions of PTX on intact and dissociated longitudinal smooth muscle from guinea pig ileum.

PTX at 1 to 10 μM produced a rapid contraction in strips of longitudinal muscle. These contractions were, at most, about 50% of the maximal contraction produced by 45 mM KCl in the same tissues. This initial peak contraction was followed over a period of minutes by a gradual relaxation of the tissue. Subsequent applications of similar toxin concentrations after this relaxation failed to elicit a further response, suggesting a form of desensitization. The contractions induced by PTX were dependent on extracellular calcium and could be reversed by 1 mM verapamil, a Ca^2+ channel antagonist.

In isolated muscles under whole cell patch clamp, PTX in the range of 1 to 10 μM induced a substantial (3 to 5 fold) increase in the resting conductance of the muscle fibers. The conductance increase was associated with a positive shift in the reversal potential and could be blocked by complete replacement of extracellular Na^+ with TEA^+ suggesting an increase in sodium and possibly potassium permeability. Like the contractile effects in muscle strips, this PTX-induced conductance was not relieved by extensive washing with toxin-free solutions. Further, even after washes with toxin-free TEA^+ solution that blocked the PTX effects, return to control saline revealed the PTX-induced conductance. At high doses, the conductance increase produced by PTX declined to a plateau, similar to the desensitization-like decrease in tension seen in muscle strips.

**ION CHANNELS: MODULATION AND REGULATION IV**

608.1 CO-FACTOR(S) FROM RAT BRAIN MODULATES THE GATING MODES OF SKELETAL MUSCLE (μ) AND BRAIN (RIIa) VOLTAGE-SENSITIVE Na CHANNELS
J.F. Potts, J. Zhou, M. Hollmann*, J.S. Trimmer*, S.A. Tomiko, J.M. DeFelice, J. Parnell, M. Hollmann, J.S. Trimmer,

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Co-expression of μ subunit α-subunit cRNA with μ subunit alone. The factor evidently modulates the time spent in the steady-state inactivated channel state, and the probability of the channel remaining in the steady-state inactivated state.

Recent studies (Zhou, J. et al., Biophys-J. (1990) and manuscript submitted) demonstrate that Na channels expressed in Xenopus oocytes from skeletal muscle μ cDNA exhibit fast and slow inactivating current components; single channel analysis reveals that individual channels may exhibit fast and slow inactivation and slow-inactivation properties. Co-expression of μ subunit cRNA from skeletal muscle μ cDNA has been shown to modulate the inactivation properties of the Na channel.

608.3 PANCURONIUM AFFECTS Na⁺ CURRENT IN CHICK EMBRYO SENSORY NEURONS. V. Magnelli*, R. Roble*, F. Maestroni*, and O. Usai

S. Z. Institute, La Jolla, Calif. 92037. Dept. of Cellular Biology & Biochemistry, SUNY at Stony Brook, Stony Brook, NY 11794.

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Anticonvulsant compounds, including MK-801, which protect against maximal electroshock seizures in animals limited sustained repetitive firing (SRF) of intracellulary recorded sodium-dependent action potentials cultured mammalian neurons. We studied the effect of temperature on the limitation of SRF by MK-801 of SRF of mouse spinal cord neurons in cell culture. Limitation occurred between 10⁻⁴ and 10⁻⁶ M (ES₅₀ 1.5x10⁻⁷ M at 37°C; between 10⁻⁸ and 10⁻³ M at 32°C (bimodal distribution); and, between 5x10⁻⁷ and 10⁻⁵ M at 23°C (EC₅₀ about 10⁻⁶ M). Thus, temperature changes altered concentration-dependence of limitation of SRF and could explain the high concentrations of MK-801 needed to see effects on Na⁺ channel isoforms.

Dorsal root ganglion neurons (DRGn) in cell culture were classified by waveform of intracellularly recorded action potentials (APs; SD: duration <2 ms; LD: duration >2 ms). Control (temperature: 37°C; 1 Hz) elicited firing in neurons with SD or LD AP waveform repeatedly with rare failures. During exposure at 37°C to a static magnetic field produced by an array of four permanent magnets of alternating polarity, the number of stimuli failing to elicit APs increased significantly and APs of both types were completely blocked in many neurons. At 32°C, SD APs failed, but less frequently than at 37°C; LD APs were not affected. No effect of the field was seen on SD APs at 23°C. Modulation of magnetic field effects on functional subtypes of DRGn with temperature may have implications in the treatment of neuropathic pain.

IMMUNOFLUORESCENT LOCALIZATION OF BANAL NUCLEAR RECEPTORS IN THE CENTRAL NERVOUS SYSTEM. W.H. Yung*. Dept. of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261.

The time-dependent hyperpolarization-activated inward current I_h (or I_o, I_o; I_a) has been observed in a variety of cell types including neurons. Several recent reports have described effects on cell excitability through modulation of I_h by intracellular messengers, and also by activation of muscarinic and adrenergic receptors.

Intracellular recordings were made from presumed dopaminergic substantia nigra compacta neurons in rat brain slices. I_h was studied using single electrode voltage clamp. The inhibitory amino acids GABA (0.3 μM), taurine (3 μM) and glycine (0.1 μM) all increased membrane Cl^- conductance and also markedly reduced the amplitude of I_h. These effects were mimicked by the GABA receptor agonist muscimol, but not by GABA agonist baclofen. The modulation of I_h by activation of neurotransmitter receptors thought to be ligand-gated Cl^- channels represents a novel mechanism for control of cell excitability. Experiments investigating the mechanism of this effect will be described.

ZINC REDUCES THE TRANSIENT OUTWARD CURRENT IN IMMATURE RAT HIPPOCAMPUS NEURONS. J. Spiegelman & P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Canada.

Previous studies have shown that endogenous zinc application has predominantly excitatory effects on mammalian central neurons. The giant GABA-mediated depolarizing potentials in immature hippocampal CA3 neurons (Ben-Ari et al. J. Physiol. 416:303-325, 1989) were recently proposed to result from an inhibitory action of Zn^2+ on the pre- and postsynaptic GABA_A-mediated receptors (Xie & Smart, Nature 349:521-524, 1991). We studied the effects of Zn^2+ and the GABA receptor agonist baclofen on the transient outward current (I_o) in immature rat hippocampal neurons. Whole-cell patch recordings from dentate granule or CA1 neurons in hippocampal slices from immature (17-13 day) Wistar rats revealed that both application of Zn^2+ (200 μM) reversibly decreased the amplitude of I_o (32%, n=7) and its rate of decay (54%), while the latency to peak I_o activation was increased. Application of baclofen (200 μM) increased the I_o amplitude (n=3). Concomitant baclofen and Zn^2+ applications that followed application of Zn^2+ alone partly offset the Zn^2+-induced decreases in I_o amplitude (n=3). These results suggest that one possible consequence of endogenous Zn^2+ release in the immature hippocampus is an increase in both pre- and postsynaptic excitability via actions on the I_o. Supported by the Ontario Mental Health Foundation and Medical Research Council of Canada.

CHLORIDE CHANNEL REGULATION IN SKELETAL MUSCLE OF AGED RAT. D. Conte Cameron*, A. De Luca*, D. Tricarico* and S.H. Bryant. Dept. Pharmacology, Faculty of Pharmacy, University of Regina, Regina, SK, Canada.

Skeletal muscle fibers of aged rats have a reduced staining chloride conductance(GC1) (De Luca et al., F1lagers Arch. 417, 1991). The present study was aimed at clarifying if perturbation of the GC1 system can explain the delayed GCI deficit of aged rats. Measurements of GC1 were made on extensor digitorum longus muscle fibers from 3-4 and 29 months old rats, "In vitro" at 30°C with standard solutions. The mean 0.55 mV of the GC1 was measured in the absence of luminal Cl- and in the presence of 10 mM luminal Cl-.

The affinity of the endogenous compound for the central benzodiazepine receptor has been extracted from rat brain tissue (Pharmacologist 30: 25, 1988). The endogenous compound was stored at 25°C, 0-4°C and -80°C for 48 hours in 70% methanol. The central benzodiazepine binding sites in homogenate of rat cerebral cortex were labeled with [3H]-flumazenil. Incubations were conducted at 0-4°C. Specific binding was measured as the difference between the [3H]-flumazenil bound in the absence and presence of 1μM clomazepam.

The affinity of the endogenous compound for the central benzodiazepine binding site, as well as its ultraviolet light absorbance decreased over time at each temperature. Storage of the compound at colder temperatures did not slow the apparent breakdown of the compound. In contrast, the benzodiazepines, diazepam and desmethylflumazenil were stable relative to the endogenous compound in 70% methanol. (Supported by NIH grant NS27265).
608.11  
BETA-ENDORPHIN MODULATES A MECHANOSENSORY CAKCAL CANNON IN STENTOR.  M. J. Marino and D. C. Wood.  Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260

Mechanical stimulation opens a population of mechanosensitive calcium channels in the contractile system of Stentor. The resulting depolarizing receptor potential can trigger an action potential and lead to a contraction of the organism. We have found that the peptide endorphin (BEND) markedly reduces the probability that Stentor will contract to mechanical stimuli. This action of BEND is naloxone resistant and dose dependant (100 nM - 10 μM). The drug is most effective immediately after application, with the contraction probability returning to control values after two hours of continued exposure. The reduction in response probability is specific to mechanically induced contractions, with no change in response produced by electric depolarization. Electrophysiological studies have shown that BEND produces a receptor potential with an amplitude without altering other passive or active membrane properties. On going voltage clamp studies are aimed at elucidating the nature of this channel modulation.

608.12  
BINDING CHARACTERISTICS OF [3H]RYANODINE TO RAT BRAIN CORTICAL MICROSONES.  L. Zimanyi and J.N. Pesah.  Department of Veterinary Pharmacology and Toxicology, University of California, Davis, CA 95616

Specific binding of [3H]ryanodine to sucrose gradient purified rat brain cortical microsomes recently has been characterized pharmacologically (Zimanyi and Pesah, Brain Res. 1991). The association of 0.5 nM [3H]ryanodine is complete within 80 min and remains stable for up to 5 hr. Dissociation of the [3H]ryanodine-receptor equilibrium complex has been examined in two ways. Addition of 1000-fold unlabeled ryanodine results in biphasic dissociation curves best fit double exponential decay which yield two dissociation rate constants. The dissociation rate constants and the pseudo first order rate constant of the association yield two dissociation constants of 0.1 and 1.7 nM for the high- and low-affinity binding of ryanodine. Dissociation of [3H]ryanodine by 1000-fold dilution of the assay medium results in monophasic dissociation with half-time of 63 min and yields a calculated Kd of 0.5 nM. Pretreatment of the microsomes with 1 μM unlabeled ryanodine, which was subsequently removed by sucrose-washing and centrifugation steps reduced the available specific binding sites for [3H]ryanodine by 50%. These results indicate an irreversible action of ryanodine on multiple sites in the brain. In general [3H]ryanodine receptors found in brain have higher affinities than those found in skeletal muscle SR. Supported by NIH grant ES05002 and BRSG 90-19 to INP.

608.13  
ALTERATIONS IN CALCIUM AND POTASSIUM CHANNEL ANTAGONIST BINDING IN CARDIOMYOPATHIC HAMSTER TISSUE.  J.A. Miller, P.A. Chichlowski and P. Ramp.  Marion Merrell Dow Research Institute, Baltimore, MD 21224

The Syrian cardiomyopathic hamster (CMH) (BIO 14.6) develops cardiac hypertrophy and congestive heart failure, possibly due to cellular calcium overload. To characterize the pathophysiology involved in this animal model we have examined the density of L-type calcium and ATP-sensitive potassium channels in 30 and 180 day old hamster heart and brain using membrane receptor binding and quantitative autoradiography (QAR). In the heart, [3H]PR950-110 binding to calcium channels was elevated 5-fold in 30 day old CMH and 3.1-fold in controls in 180 day old CMH. In contrast, [3H]glibenclamide ([3H]GLI) binding to cardiac potassium channels was unaltered in 30 day old animals but was decreased to 64% of control levels in 180 day old CMH. In brain membranes there was no significant difference in the binding density of either ligand between groups or between ages. However, QAR revealed a 4.1-fold increase in [3H]GLI binding in frontal cortex of 30 day old CMH versus the same age control. There was no difference in [3H]GLI binding in frontoparietal or striate cortex or in cerebel- lum or brainstem. Further QAR analyses will include other brain and heart regions with both [3H]PR and [3H]GLI binding. These findings suggest distinct differences in regional levels of potassium and calcium channels in CMH that may be related to the progression of the disease.

608.14  

The general anesthetic Propofol (PR) reduced reversibly K+ currents in voltage clamped PC12 cells. In control conditions, reduced active QAR was used, followed by a two exponential terms, with n2 and n1 kinetic- ics, whereas, when PR was applied extracellu- larly, the n2 component only gave the best fit. PR 5x10^-6 to 1x10^-5 decreased currents amplitude in a concentration dependent way, both independently of holding potential (Vh). K+ currents blocked by 30% and 60% PR block was observed, when the above concentrations were employed. Single channel outside-out experiments (Vh= -50 mV) revealed 2 different K+ channels, with unitary con- ductances of 10 and 20 pS. PR 5x10^-5 to 1x10^-4 decreased the open probability of the bigger channel, but did not affect the smaller one. At higher concentrations channels were fully blocked. These results might suggest either different sensitivities or different blocking mechanisms for the two channels.

608.15  
INTERACTIONS OF POLYAMINES WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR IN RAT BRAIN.  B.D. Kuzros, C.R. Mantione and E.D. London.  Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21222

A previous report indicated the presence of specific recognition sites for polyanines on the nicotinic acetylcholine receptor (nACH-R) of Torpedo electric organ (Anisz et al., J. Neurochem. 54:764, 1990). Our, therefore, were interested in whether polyanines, which modulate activity of the N-methyl-D-aspartate receptor, also interact with nACH-R. In this regard, we tested the effects of various endogenous and synthetic polyanines on the binding of [3H]nicotinylicarbamylcholine and [3H]mecamylamine ([3H]MEC), which label the acetylcholine recognition site and the open conformation of the nACH-R (London, E.D. et al., Soc. Neurosci. Abstr. 15:64, 1989, Banerjee, S. et al., Biochem. Pharmacol., 40:2105, 1990), respectively. Although the polyanines had no effect on the binding of [3H]nicotinylicarbamylcholine, they inhibited the binding of [3H]Mecamylamine at submicromolar concentrations. The rank order of potencies of the compounds tested was as follows: spermine > spermidine > 1,10-di- amino-1-decanone > putrescine > diethylenetriamine. The sequence is similar to the rank order of these substances in inhibiting the binding of [3H]putrescine, a radiog梁 for polyanine binding sites in brain (Mantione, C.R. et al., Eur. J. Pharmacol. 180:393, 1990). The present findings suggest that polyanines in brain may modulate the activity of nACH-R through recognition sites on the receptor.

608.16  

Previous studies in our laboratory have shown an anticholinergic effect of quinidine sulfate, an antirhythmic compound, caracte­ rized by producing a slight increase in tone, at low doses, and a decrease in tone and rhythm when applied at high doses on the isolated intestine of guinea pigs. Segments of intestine from 300 g male guinea pig were prepared for isometric recording. Quinidine inhibited the con traction elicited by acetylcholine. This effect can also be observed with histamine and barium chloride. These effects are calcium non-dependent as they can be observed in low calcium solutions. Quinidine, an anticholinergic, an antihistaminic, and an antibi­ arium effect of quinidine, calcium non-dependent.
609.1
REGULATION OF HIGH-AFFINITY CHOLINE TRANSPORT IN THE NEUROBLASTOMA CELL LINE LA-N-2. A. Lambros*, S. Goddard*, and R.J. Ryrett. Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, Canada. N6A SCI. The sodium-dependent, high-affinity choline transport protein has not been purified or characterized biochemically and very little is known about its long-term regulation. We have observed that transfer of cholinergic neuroblastoma LA-N-2 cells into serum-free media results in enhanced high-affinity choline uptake (HACU) activity accompanied by proportional increases in acetylcholine (Ach) synthesis, but not choline acetyltransferase (ChAT) activity. Maximal increases in HACU (approximately 7-fold) were achieved after four days in SF-N2 media. Enhanced HACU was characterized by an increase in the maximum velocity of transport (Vmax) for the high-affinity carrier with no significant change in the affinity (Km) of the carrier for substrate. In the presence of cycloheximide or α-amanitin, increases in HACU activity were substantially reduced. This suggests that transfer of LA-N2 cells into SF-N2 media results in increased synthesis of mRNA transcript for the high-affinity carrier which is effectively translated into new transport protein. Our findings indicate that LA-N-2 may serve as an effective model for studying regulation of HACU in culture. (Supported by MRC Canada and The Upjohn Company.)

609.2
ANALYSIS OF REGULATORY ELEMENTS IN THE 5' FLANKING REGION OF THE L-DOPA-DECARBOXYLASE GENE. K. Boda, T. Klimasewski* and P. M. Salvari. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010. We have analyzed the 5' flanking region of the Drosophila L-dopa decarboxylase gene (LDC, E.C.2.3.1.6) for the presence of cis-regulatory elements. DNA fragments containing various lengths of flanking DNA were fused to the E. coli lacZ gene used to transform Drosophila. The histochemical localization of β-galactosidase expression was used to evaluate the ability of different fragments to direct lacZ expression. A 7.4 kb flanking sequence was found to direct β-gal expression in specific somal and synaptic layers of the optic lobe and other cephalic structures. The pattern of β-gal staining corresponded closely to the distribution of endogenous ChAT protein. In contrast, a construct containing only the proximal 3.3 kb of 5' flanking DNA resulted in a qualitatively and quantitatively different expression pattern. In general, a more restricted pattern of expression was observed which appeared to be a subset of the structures seen with the 7.4 kb construct. Further deletion to the proximal 1.2 kb of flanking DNA led to an even more restricted pattern. Our results indicate that both qualitative and quantitative regulatory elements are present in the 5' flanking DNA and these elements distinguish subsets of cholinergic neurons. We have also fused the 5' flanking DNA to wild type ChAT cDNA, and used these constructs to transform Drosophila stocks carrying a temperature sensitive ChAT allele. The 7.4 kb cDNA transformants had higher levels of wild type ChAT activity than either the 3.3 or 1.2 kb constructs. All three constructs were able to rescue mutant flies from paralytic and lethargy phenotypes. (Supported by M.R.C. of Canada.)
609.5

Heptyl-phosystigmine (HEP) is a long-acting physostigmine (PH) derivative which inhibits acetylcholinesterase (AChE) and elevates acetylcholine (ACh) concentration in homogenates of the cerebral cortex and striatum (De Sarno et al. 1989). This study examined the effect of systemically administered HEP on extracellular ACh using a microdialysis technique. The effect did not incorporate additional AChE inhibitors in the probe perfusate. Basal ACh concentration in the cortical dialysate of conscious rats was 5.7 ± 0.3 nmol/100 g after 1 mg/kg (i.p.) and by 1200% after 5 mg/kg (i.p.) HEP. The observed HEP effects were not altered by the presence of atropine (0.1 μM) in the dialysis probe perfusate. In contrast to the increases noted in cerebral cortex, microdialysis in the striatum did not reveal increased extracellular ACh in response to either dose of HEP. When the dialysis probe contained PH (10μM), the 5 mg/kg HEP injection decreased extracellular ACh by 20%, despite an overall 60% increase in ACh content of the striatal homogenate. These results do not support a necessary correlation between the ACh content in brain homogenate and the extracellular space. They further suggest marked regional variation in the response to cholinesterase inhibition.


609.7

Sarin, a highly toxic organophosphate administered at near LD50 dose causes consistent brain lesions, the mechanism of which is not fully understood. The present study was designed to evaluate the immediate and long-term neuropathology of a single LD50 dose of sarin, and to elucidate the role of excessive acetylcholine in creating these lesions. The neuronal lesions were compared to other cholinesterase (ChE) inhibitors. Rats surviving LD50 dose of sarin, soman or DFP were sacrificed at 1, 7, 30 and 90 days after administration, and their brains were histologically evaluated. While brains of all soman-injected rats were severely damaged, only 70% of the sarin and none of the DFP injected rats were affected. Although the general location of brain neuropathology following sarin resemble that of soman, sarin induced lesions were variable and uniquely affected nerve fibers whereas soman initially damaged only cell-bodies. The different patterns of brain lesions of soman, sarin and DFP indicated a distinctive mode of action for each of them, not mediated solely by ChE inhibition.

609.8
IN VIVO AND IN VITRO INTERACTION OF CARBAMATES WITH QUATERNARY OXIMES G. Cohen*, E. Cohen#, R. Amiati, IIBR, Ness Ziona 70450, and Agrocl. Faculty The Hebrew Univ., Rehovot 76100, ISRAEL.

The antidotal treatment employed in the case of poisoning by AChE inhibitors usually consists of a combination of atropine and a quaternary oxime (e.g. toxogonin or 2-PAM). However, in the case of carbaryl poisoning toxogonin increases its toxicity by 3.5 fold. AB-8 and AB-13 are quaternary oximes previously shown to be potent antidotes against poisoning by various AChE inhibitors. In the case of carbaryl poisoning in mice, no survival was obtained with either AB-13 (50 mg/kg, i.v.) or toxogonin (12.6 mg/kg, i.v.) in combination with atropine (0.2 mg/kg, i.m.). However, AB-8 (50 mg/kg) protected 80% and 60% of the animals against 3 and 4xLD50 of carbaryl, respectively. In vitro reactivation studies with purified eel AChE showed that toxogonin and AB-13 are 1500 and 500 fold more active than AB-8, respectively. It is, therefore, suggested that oximes which are potent AChE reactivators should be excluded from the antidotal treatment of carbaryl poisoning. In addition, the mechanism of action of AB-8 may not be solely related to either AChE reactivation or anticholinergic activity.
610.1 EFFECTS OF SOLUTION STRUCTURE AND DYNAMICS ON THE FUNCTION OF A SERIES OF NICOTIC CHOLINERGIC AGONISTS. K.A. McGroddy, A.A. Carper, M.M. Tibbets & R.E. Oswald. Dept. Pharmacology, Cornell Univ. Health Sci. Ctr. Induction of the binding of a ligand to its receptor, which leads to receptor activation, is dependent upon the structure and dynamics of the receptor both in solution and in the binding site. We have synthesized two classes of chemically similar cholinergic ligands based on the parent compounds shown below in order to determine the contributions of the receptor structure and dynamics to the ability of these compounds to bind to and activate nicotinic acetylcholine receptors (nAChRs).

The structural and dynamic variations of these compounds have been compared structurally and theoretically, and 2D-1H and 19F NMR studies have shown that the TORDO compounds exist in two stable solution structures of different energy, with a high barrier to interconversion. Only the higher energy conformer could achieve the correct conformation for binding to the receptor. The more stable TORDO structure differs significantly from the rigid PIP structure and this can account for the difference in affinity between these two compounds. Molecular dynamics simulations have been carried out using the CHARMM program. The systems have consisted of one ligand in a box of TIP5P water with periodic boundary conditions. Trajectories of each of these compounds have been simulated and analyzed in order to study the effect of solvation on the structures and dynamics of these molecules and to determine the relative contributions of enthalpy and entropy to the interaction of these drugs with nAChRs. These studies indicate that the solution structure and dynamics of a compound can make an important contribution to the binding affinity of a ligand to its receptor.


BZQ, a synthetic neuromuscular blocking agent (J. Am. Chem. Soc., 72: 2661, 1950), has been proposed to block the physostigmine (PHY) agonistic action on the nAChR complex from Torpedo (Okano et al., in press). In this work, further characterization of the BZQ effects at the AChR was done by electrophysiological studies in Rana pipiens muscles and ligand-binding assays in Torpedo membranes. Voltage-clamp was performed on sartorius muscle-sciatic nerve preparation, and channel-activated single channel currents were recorded from interosseous fibers using cell-attached mode. The nAChR response to ethidium binding in the presence of competing NCIs showed that ~90% of the total amplitude of the double-exponential decay process was characterized by a short-lifetime component of 1.6 ns. This lifetime is attributed to ethidium in buffer indicating that ternary complexes, in which ethidium and another NCI are simultaneously bound, do not form (Herz, et al. JBC 262, 7226, 1987). Dissociation rates induced by addition of FC, TIPMP and morpholine were similar, but distinct from chlorpromazine and quinuclidinylbenzilate (QX-222). Ethidium bound to the presence of competing NCIs could be precipitated easily from other oocytes, the same mutant α subunits, having one or the other of the cysteines replaced by serine, for additional detailed studies. Both of the mutant α subunits assembled with ACh, which can be competed by ethidium when expressed in Xenopus oocytes. These data can be discussed in terms of competitive mechanisms and subsite models. Supported by AHA Texas Affiliate 89G-401.

610.4 EXPRESSION OF ALPHA-BUNGAROTOXIN-INSENSITIVE FUNCTIONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN XENOPOUS OCYTOPES. V. M. Gehle and K. Sumikawa. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717.

A conserved feature of many ligand-gated ion channels, including the muscle and neuronal ACh receptors, GABA receptor, and glycine receptor is a pair of cysteine residue separated by 13 amino acids in the extracellular portion of the protein. In the Torpedo AChR α subunit, this pair has been demonstrated to form a disulfide bond. This disulfide bond may be important for receptor function because removal of the two cysteines from the AChR α subunit was demonstrated to affect the ligand binding and ACh receptor expression (Mishina et al., 1985, Nature 313: 364-369). That report indicated the cysteines are critical for AChR function, but did not precisely define the role they play. We have created the same mutant α subunit, having one or the other of the cysteines replaced by serine, for additional studies. Both of the mutant α subunits assembled with ACh, which can be competed by ethidium when expressed in Xenopus oocytes. These data could not be blocked by application of α-BuTX. Thus, formation of a disulfide bond between Cys128 and Cys142 of the Torpedo AChR α subunit is necessary for α-BuTX binding, but not ACh binding and ion channel function.

610.5 EFFECT OF SUBSTITUTION OF TORPEDO ACETYLCHOLINE RECEPTOR (α7β7γγ) α-SUBUNIT RESIDUES WITH SNAKE RESIDUES ON BINDING OF α-BUNGAROTOXIN (α-BTX). D. L. Donnelly-Roberts*, Y. Chaturevedi* and T. L. Lentz. Dept. of Biology, Yale University School of Medicine, New Haven, CT 06510.

A fusion protein consisting of the Trp E protein fused to residues 166-211 of the Torpedo AChR γ subunit was produced in E. coli. Using site-directed mutagenesis, a fusion protein consisting of Torpedo AChR γ subunit (gift of Toni Claudio) was digested with restriction enzymes to yield a 137 base pair fragment encoding residues 166-211. This fragment was fused to the 3’ terminus of the Trp E gene using a pATH10 expression vector. Whereas Torpedo AChR α subunits yielded high affinity, AChR from snakes are toxin-resistant (Burden 1990). This fragment was fused to the 3’ terminus of the α7β7γγ receptor subunit. The resulting peptides were expressed in oocytes using a eukaryotic cell line in order to induce expression of the peptides. Black Syrian hamster oocytes electroplated with the indicated Kd values: chlorpromazine (0.15 μM), TIPMP (0.52 μM), PCP (0.57 μM), QX-314 (20.1 μM) and QX-222 (494 μM). Indirect Hill coefficients for all NCIs were 1.0. Analysis of ethidium’s excited state lifetime in the presence of competing NCIs showed that >90% of the total amplitude of the double-exponential decay process was characterized by a short-lifetime component of 1.6 ns. This lifetime is attributed to ethidium in buffer indicating that ternary complexes, in which ethidium and another NCI are simultaneously bound, do not form (Herz, et al. JBC 262, 7226, 1987). Dissociation rates induced by addition of PCP, TIPMP and morpholine were similar, but distinct from chlorpromazine and quinuclidinylbenzilate (QX-222). Ethidium bound to the presence of competing NCIs could be precipitated easily from other oocytes, the same mutant α subunits, having one or the other of the cysteines replaced by serine, for additional studies. Both of the mutant α subunits assembled with ACh, which can be competed by ethidium when expressed in Xenopus oocytes. These data could not be blocked by application of α-BuTX. Thus, formation of a disulfide bond between Cys128 and Cys142 of the Torpedo AChR α subunit is necessary for α-BuTX binding, but not ACh binding and ion channel function.

610.6 A MUTANT GAMMA SUBUNIT OF TORPEDO ACHR CAUSES DEGRADATION OF CO-EXPRESSED NORMAL SUBUNITS. K. Sumikawa and V. M. Gehle. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717.

Using site-directed mutagenesis, we changed the asparagine residue at the conserved N-glycosylation site (position 141) on the γ subunit of the Torpedo acetylcholine receptor (a-BTX) binding domain. When these mutant subunits were expressed in Xenopus oocytes along with non-mutant α, β, and δ subunits (my-AChR), very little α-BTX binding activity was seen. Our results indicate that the γ subunit is necessary for α-BuTX binding, but not ACh binding and ion channel function.
610.7 MODULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR ION CHANNEL FUNCTION BY THE M4 TRANSMEMBRANE DOMAIN. L. L. Y. L. Lee* and M. G. McIntyre. Dept. of Biochem. & Biophysics, Univ. of California, Davis, CA 95616. The role of M4 transmembrane helices in nictinic acetylcholine receptor (nAChR) channel function was investigated by studying the functional consequences of site-specific mutations of cysteine residues in the M4 helices of Torpedo californica AChR α and β subunits using Xenopus laevis oocytes injected with in vitro synthesized [35S]RNA transcripts. The mutation of Cys18 to Trp increased the AChR normalized channel activity (measured as the ACh-induced conductance per femtomole of surface m-muscarinic acetylcholine receptor binding sites) by more than 40 fold. In contrast, the mutation of Cys451 to either Ser or Trp decreased the normalized channel activity by about 50%. The surface [3H]a-BGT binding assay and the Western blot analysis of membrane preparation revealed that these mutants had the same expression level as the wild type receptor. These mutant AChRs displayed a linear current-voltage relationship with a reversal potential of around 0 mV similar to that of the wild type receptor, suggesting that these mutations did not alter the selective permeability of the AChR channel for cations. Moreover, these mutations did not change other AChR functional properties such as agonist binding ability, the slow phase of desensitization, and blockade by specific nAChR antagonists. These results suggest that M4 transmembrane domains can modulate AChR ion channel function, possibly by altering channel gating.

(Supported by USPHS Grant NS 22941)

610.9 MUSCLE ACTIVITY SUPPRESSES EXPRESSION OF EMBRYONIC-TYPE NICOTINIC ACETYLCHOLINE RECEPTOR (α6b) GENES VIA A CYCLIC AMP SECOND MESSENGER SYSTEM. K. G. Chahine*, I. Staple, L. L. Mark, L. Vanvorse, W. Waltse* and D. Goldman. Mental Health Research Institute and the Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109. During development of the neuromuscular junction there is a switch in expression of α6b genes from an embryonic (α6βδγγ) to an adult-type (α6β3δ) phenotype. Embryonic-type genes are expressed throughout the muscle fiber prior to innervation. However, after innervation, extrajunctional expression of these genes is suppressed by muscle activity. We have determined a mechanism by which this repression occurs by using a rat primary muscle culture system that responded to muscle activity (induced by stimulation with extracellular electrodes) in a manner similar to that observed in vivo. We found that increasing intracellular cAMP reversed the effects of muscle activity on embryonic-type nAChR gene expression. Genes not regulated by muscle activity, such as those encoding the nAChR ε-subunit and creatine kinase, were not induced by increasing cAMP. Consistent with these results was the finding that muscle inactivity, induced in vivo by denervation or in vitro by muscle denervation, caused an increase in cAMP and decreased sensitivity to a-gonists.

610.10 STABILIZATION OF ACETYLCHOLINE RECEPTORS IN NEURAL RAT MUSCLE CULTURES BY CYCLIC AMP ANALOGUES J. O'Malley*, M. L. Shelanski & J. L. Rubin. Department of Pathology, Columbia Univ., New York, NY 10032. The stabilization of AChRs at the neuromuscular junction (nmj) occurs at birth when the receptor's half life increases from the fetal value of approximately 1 day to the mature value of 7-10 days. Denervation has been shown to partially reverse AChR stabilization and results in the expression of two populations of AChR, one with a rapid turnover of 1 day (RACRs) and another with a longer turnover of approximately 4 days (RACRs). Recently it has been shown that agents which elevate cAMP can stabilize the RACRs at the nmj of denervated muscle to a level similar to that of adult-type AChRs found in normal muscle (t1/2-7 days). We wanted to determine if these agents were also able to stabilize AChRs in aneural muscle cell cultures, which normally only express fetal-type AChRs with a half life of 1 day. We report that cells treated for 4 days with 250 μM 8-(4-chlorophenylthio)-cAMP, 1 mM Dibutyryl-cAMP or 10 μM forskolin express two populations of AChR, one with a half life of 1 day and another with a half life of approximately 12 days which constitute 75% and 25% of the total receptor population respectively. However if the period of treatment was shortened to less than 4 days the stable AChR population was not seen. We would be interesting to know if the receptors which have a half life of 12 days share other properties, such as channel kinetics, with adult-type AChRs in normal muscle and if they are thus physiologically equivalent to the stable AChRs of adult muscle.

610.11 TRANSCRIPTIONALLY-MEDIATED REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN HUMAN CLONAL CELLS. Ronald J. Lukas and Linda Lucero. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013. Chronic treatment of TE671/RD human cells with phorbol 12-myristate-13-acetate (PMA) or with nicotinic agonists induces upregulation of muscle-type nAChR alpha and beta subunit genes (α6b). Only the PMA effect is blocked by nerve growth factor, or dibutyryl cyclic AMP) also induces in control levels of α6b mRNA. Thus, chronic PMA treatment can activate transcription of muscle-type and neuronal α6b genes.

610.12 UP REGULATION OF ACETYLCHOLINE RECEPTORS IN THERMAL INJURY J. M. Kard & J. A. Martyn*. Dept. Anesthesia, Harvard Medical School and Anesthesia Services of Massachusetts General Hospital and Shriners Burns Institute, 51, Blossom Street, Boston MA 02114. Severe burn injury results in dramatic changes in human pathological physiology which can result in many previously therapeutic difficulties. Important sequelae in burn patients include weakness and respiratory muscle failure and an altered response of general nicotinic acetylcholine receptors (α6b) to neuromuscular relaxant drugs used clinically. In thermally injured rats using a single, saturating concentration of [125I] alpha-bungarotoxin (α-BGT), we have demonstrated an increase of α-BGT binding to α6b receptors extracted from gastrocnemius muscle with respect to control (P=0.05 ANOVA one way). One explanation for the observed increase in α6b binding is an up-regulation of α6b gene expression which might occur in vivo. We propose that the altered responses to neuromuscular relaxant type drugs are due, in part, to a decrease in affinity which promotes up-regulation of the α6b.

The nicotinic acetylcholine receptor (nACHR) is highly phosphorylated on tyrosine residues in vivo. Previous studies have shown that denervation of muscle decreases tyrosine phosphorylation of the nACHR suggesting that innervation of muscle regulates phosphorylation of the nACHR. To examine more directly the role of innervation in the stimulation of tyrosine phosphorylation of the nACHR, we have used neuron-muscle cell cultures. Immunocytochemical staining with anti-phosphotyrosine antibodies show that nACHR clusters at synapses between nerve and muscle contain high levels of phosphotyrosine. In addition, neuronal innervation of muscle cell cultures increased tyrosine phosphorylation of the nACHR as analyzed by immunoblot techniques with anti-phosphotyrosine antibodies or by directly examining the Tg- incorporation into the nACHR. Recent results have suggested that the neuronal extracellular matrix protein, agrin, which is thought to be released from the neuron and induce clustering of the nACHR, may be the factor from the nerve that regulates tyrosine phosphorylation. These results suggest that tyrosine phosphorylation may mediate nerve induced clustering of the nACHR at the neuromuscular junction.


Tyrosine phosphorylation has been shown to regulate the functional properties of the nicotinic acetylcholine receptor (nAChR). The level of tyrosine phosphorylation of nAChR is regulated through a balance between tyrosine phosphorylation by protein kinases and phosphotyrosyl dephosphorylation by protein phosphatases. We have purified and characterized a protein tyrosine phosphatase that dephosphorylates tyrosine phosphorylated nAChR from Torpedo electroplax. The [32P]-labeled tyrosine phosphorylated nAChR was used as a substrate to monitor the enzyme activity during purification. The protein tyrosine phosphatase activity was purified using three consecutive cation-exchanger columns (phosphocellulose, S Sepharose Fast Flow, BioRes 70), followed by affinity matrices (p-aminophenylphosphonic acid-agarose and phosphotyrosyl-Sepharose 4B). The enzyme activity was purified to homogeneity, with an overall purification of 25,000-fold and a yield of 20%. The purified enzyme had an apparent molecular weight of 43 kDa on SDS polyacrylamide gels and migrates as a monomer during Superose 12 chromatography. It has a neutral pH optimum and a specific activity of 18.8 µmol/min, with a Kd of 4.7 µM for tyrosine phosphorylated nAChR. The phosphatase was specific for tyrosine phosphorylated nAChR; it showed no activity towards the nAChR phosphorylated on serine residues by CaM-dependent protein kinase. The tyrosine phosphatase had unique sensitivities to inhibitors and metal ions, suggesting that it may be a novel protein tyrosine phosphatase that dephosphorylates the nAChR and other neurotransmitter receptors. We have recently obtained protein sequence of tryptic peptides of the purified phosphatase and are currently attempting to isolate cDNA clones of the protein tyrosine phosphatase.

610.16 STAUROSPORINE INHIBITION OF RECOVERY FROM DESENSITIZATION IS REVERSED BY CALCIUM AT SNAKE TWITCH FIBER ENDPLATES. J.C. Stavenga and R.L. Huganir, Dept. of Pharmacology, University of Washington School of Medicine, Seattle, WA 98195. We showed previously, at snake nicotinic acetylcholine receptors, that the recovery of receptor sensitivity to agonist following desensitization was inhibited by pretreatment with the protein kinase inhibitor, staurosporine (Harwick & Parsons (1990) Neurosci Abstr 16:205). These findings suggested that recovery of the desensitized receptor involved protein phosphorylation. We demonstrate here that elevation of the external calcium concentration during agonist application, which results in an increase in calcium influx through agonist-activated endplate channels, reversed the inhibition of the rate of recovery produced by staurosporine. Garter snake (Thamnophis) twitch fibers were maintained in an isotonic potassium propionate solution and individual endplates voltage clamped to +30 mV. Desensitization was induced by microinjection of 540 nM carbachol containing either 1 nM or 10 nM CaCl2 for 2-3 minutes. Recovery was determined by measuring the reappearance of spontaneous miniature endplate currents (MEPCs) during the wash period. Elevation of the external calcium concentration during the carbachol application completely antagonized the inhibition of recovery produced by pretreatment with 0.5 µM staurosporine. Elevation of external calcium in the absence of agonist increased the frequency of MEPCs but did not alter their amplitude or decay time course in either control or staurosporine treated fibers. Elevation of external calcium also increased the rate of desensitization onset of the initial calcium-induced current in both control and staurosporine-treated fibers. However, the subsequent acceleration of desensitization seen with a second application of carbachol was less in staurosporine-treated fibers as compared to controls. (Supported by NS 08580 (JCH), NS 25973 (RLP) and an MDA grant (RLP)).

Recent molecular cloning studies have demonstrated the existence of five acetylcholine muscarinic receptor genes differentially distributed and pharmacologically distinct. We examined the ontogenetic expression of m1-m5 receptor gene by the Polymerase Chain Reaction (PCR) method RNA from foetal and newborn rat brain regions or from primary cultures generated from the various CNS areas was amplified by the PCR using specific sets of primers designed to recognize each individual receptor subtype. The cycle conditions were optimized for PCR amplification of receptor reverse transcribed RNA and that of hypoxanthine phosphoribosyltransferase which was included as a constitutively expressed internal standard. The signals were quantified by densitometric analysis using a Phosphorimager. This technique revealed that each receptor subtype displays a unique pattern of expression during ontogeny. m3 and m5 are apparently expressed around the time of birth, m2 and m4 can be detected as early as E13. The nature of which requires to be examined further.

ALTERATIONS IN CORTICAL MUSCARINIC RECEPTOR SUBTYPE mRNA FOLLOWING KAINIC ACID LESION OF RAT NUCLEUS BASALIS MAGNOCELLULARIS. Z. Zerg and J. van Cessen. Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

The cholinergic deficit seen in Alzheimer's disease (AD) is associated with cell loss in the nucleus basalis magnocellularis (NBm). Previous studies have shown that a major portion of the cholinergic projection to the cerebral cortex is derived from the NBm. This cholinergic input can be destroyed in rat by kainic acid microinjection into the NBm. The present results are compatible with a role of cholinergic receptors in the regulation of forebrain development.
CHRONIC SCOPOLAMINE ADMINISTRATION DIFFERENTIALLY ALTERS EXPRESSION OF m1-MUSCARINE ACETYLCHOLINE RECEPTOR GENE IN ALZHEIMER'S DISEASE BRAIN AND AGE-MATCHED CONTROL BRAIN.

B. Thorne, P.R. Potter, Dept. of Anesthesiology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx NY 10467.

The effects of cholinergic agonists on the evoked release of [3H]acetylcholine (ACh) were studied in male Sprague-Dawley rats in which hippocampal cholinergic terminals were lesioned with the neurotoxin AF64A (ethylcholine mustard aziridium, 2 nmol/ventricle). AF64A treatment causes reductions in cholinergic markers similar to those in Alzheimer's disease. Two weeks after AF64A infusion, cholinergic system activity was decreased by more than 40%. ACh release was evoked from superfused hippocampal slices by electrical stimulation (1 or 2 Hz, 2 min). In control tissues, ACh release was enhanced by nicotine (0.4-40 μM). In slices from AF64A treated rats, the dose response curve for nicotine was shifted to the left (EC50 3.5 μM). The effects of nicotine were blocked by the antagonist dihydro-beta-erythroidine (10 μM). In contrast, the dose response curve for inhibition of ACh release by the cholinergic agonist oxotremorin, was shifted to the right in slices from AF64A-treated rats compared to controls. These results suggest that functional changes have occurred in hippocampal ACh receptors as a result of lesioning cholinergic inputs, with an increase in nicotinic receptor number or sensitivity, and a decrease in muscarinic receptor number or responsiveness.

CHOLINERGIC RECEPTOR CHANGES IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE.

R. Thorne and P.E. Potter, Dep. of Anesthesiology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx NY 10467.

The effects of cholinergic agonists on the evoked release of [3H]acetylcholine (ACh) were studied in male Sprague-Dawley rats in which hippocampal cholinergic terminals were lesioned with the neurotoxin AF64A (ethylcholine mustard aziridium, 2 nmol/ventricle). AF64A treatment causes reductions in cholinergic markers similar to those in Alzheimer's disease. Two weeks after AF64A infusion, cholinergic system activity was decreased by more than 40%. ACh release was evoked from superfused hippocampal slices by electrical stimulation (1 or 2 Hz, 2 min). In control tissues, ACh release was enhanced by nicotine (0.4-40 μM). In slices from AF64A treated rats, the dose response curve for nicotine was shifted to the left (EC50 3.5 μM). The effects of nicotine were blocked by the antagonist dihydro-beta-erythroidine (10 μM). In contrast, the dose response curve for inhibition of ACh release by the cholinergic agonist oxotremorin, was shifted to the right in slices from AF64A-treated rats compared to controls. These results suggest that functional changes have occurred in hippocampal ACh receptors as a result of lesioning cholinergic inputs, with an increase in nicotinic receptor number or sensitivity, and a decrease in muscarinic receptor number or responsiveness.

The third intracellular (i3) loop of each of the subtypes of muscarinic receptor is unique and was chosen to be used as an antigen to raise antisera selective for each of the subtypes. The cDNA encoding most of the i3 loops of the m1, m2, m4, and m5 receptors were subcloned into expression vectors (either PRIT or PET). The areas close to the putative membrane-spanning regions 5 and 6 were avoided as these areas do not have the uniqueness found in the remainder of the i3 loop. The plasmids were sequenced to verify orientation and protein was produced by transforming E. Coli. Proteins were purified by a variety of methods (affinity chromatography, isoelectric focusing, size exclusion chromatography) to obtain full-length or nearly full-length proteins representing the i3 loop of a given subtype of muscarinic receptor. These proteins were injected into rabbits and sera were tested by immunoprecipitation precipitating subunits from cells transfected with the cDNA encoding a single muscarinic receptor subtype. Following 2 to 5 boosts with antigen, the antisera were able to quantitatively immunoprecipitate the expected subtype with no precipitation of any of the other subtypes. Additionally, for the m3 receptor, a short (18 amino acid) synthetic peptide corresponding to the sequence of the C-terminus was coupled to large carrier proteins and utilized as an antigen. Antisera obtained quantitatively precipitated m3 receptors with no precipitation of any other subtype of muscarinic receptor. Supported by GM31155 and NS26934, PDF and ADRDA.


Using a set of subtype-selective antagonists we have generated against each of the five cloned muscarinic receptor subtypes (Li et al. accompanying abstract), the density and distribution of each of these subtypes has been determined in a number of tissues and following a number of experimental paradigms. Thus, the densities of each of the receptors in 7 areas (cortex, hippocampus, striatum, olfactory tubercle, thalamus/hypothalamus, pars/medulla, and cerebellum) of rat brain and 16 areas of human brain have been determined. Likewise, the densities of the subtypes were determined in several peripheral tissues of the rat (lung, stomach, bladder, parotid) as well as in several widely used clonal cell lines (SK-N-SH, 132-1N1). Additionally, the effects on receptor densities of chronic administration of the nonselective muscarinic cholinergic receptor antagonist, atropine, were determined in rat cerebral cortex/insular hippocampus. Also, the ontogenetic profiles of each subtype were determined in rat forebrain. Furthermore, the effects of age on the density of muscarinic receptor subtypes in the rat hippocampus were studied. It will be useful to compare these results to those obtained previously using radioligand binding techniques. Supported by GM31155 and NS26934, PDF, and ADRDA.


In the present experiments, afferent input to the hippocampus (HPC) and cingulate cortex (CC) of adult female rats was removed unilaterally by aspiration lesion of the rostral supracallosal stria/cingulum bundle and fimbria/fornix. Ten days after the lesion cholinergic receptors were examined in the HPC and CC. Nicotinic receptors were measured using [3H]cytisine binding at near saturating concentrations (3.7nM). No significant differences in nicotinic binding between control and lesioned sides was found in the HPC, suggesting that few, if any, of these receptors are localized on cholinergic terminals. In contrast, nicotinic sites in the CC were significantly decreased (28%; p<0.01) on the lesioned side. This decrease could reflect a loss of receptors on cholinergic or non-cholinergic afferents, such as dopaminergic. Total muscarinic receptors in the HPC (measured using 1mM [3H]-QNB) were increased slightly (19%; p<0.05) on the lesioned side. More interestingly, measurements of each receptor subtype (m1-m5) using selective antisera revealed marked increases in m4 (81%; p<0.01) and m5 (31%; p<0.05) receptors and a decrease (27%; p<0.05) in m2 receptors. The direction of these changes may indicate the pre- or post-synaptic localization of these receptor subtypes. Supported by GM31155 and NS26934, PDF and ADRDA.

611.18 INTERACTIONS OF HALOPERIDOL WITH MUSCARINIC RECEPTORS. R.J. Smyth*, G.R. Luthin†, T.J. Shieky*, Department of Pharmacology, Philadelphia College of Pharmacy & Science, Phila. PA 19104. Department of Pharmacology, Johns Hopkins University, Baltimore, MD 21205.

Chronic administration of the antipsychotic drug haloperidol is often accompanied by extrapyramidal motor disturbances and dyskinesias. Other researchers have demonstrated that chronic haloperidol treatment causes an up-regulation of muscarinic cholinergic receptors. Comparison of primary amino acid sequences reveals a high degree of homology between anti-haloperidol monochlorinated doxycycline, and putative transmembrane domains of dopaminergic and muscarinic receptors. Our studies of secondary structure of these membrane bound proteins also predict structural similarity between dopaminergic and muscarinic receptors.

We therefore hypothesized the presence of a specific haloperidol binding site on muscarinic receptors, capable of interfering with agonist and antagonist binding, which may contribute to the muscarinic effects observed following haloperidol treatment.

Radioligand binding to muscarinic receptors was examined using [3H]- oxotremorine-M for high affinity agonist binding and [3H]-N-methylscopolamine for antagonist binding. Haloperidol inhibited both agonist and antagonist binding in rat cerebral cortex, and heart membranes with IC50 values in the low nanomolar range. Binding was unaffected by addition of GTP. Inhibition occurred over approximately a ten-fold concentration gradient, indicating possible positive cooperativity. A concentration-dependent effect of haloperidol on association kinetics was also observed. Together these data suggest that haloperidol inhibition of muscarinic ligand binding may not be explained by simple competitive interactions. Supported by USPHS Grant NS-26040 to JTS and by NS-23006 and a Scottish Rite Schizophrenia Grant to GRL.
612.1 HIGH pH INCREASES THE POTENCY OF PHOSPHONATE SUBSTITUTED COMPETITIVE ANTAGONISTS AT NMDA RECEPTORS. M. Benjamin and M. L. Mayer. Section of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

Structure-activity relationships have shown that α-phosphono-amino acids are more potent competitive antagonists at the NMDA receptor than α-sulfonated analogs with carbamate or triazole groups. The phosphonate group can have a maximum of two negative charges and therefore its analogs have only one negative charge, suggesting that the higher potency of α-phosphono compounds could result from an electrostatic interaction between the NMDA receptor and the NMDA molecule. Because the pK_a of one phosphonate oxygen in those compounds is ≈ 7.8 (Chenard et al., 1990; L. Chen, 33:1077), the potency of ionic species is strongly influenced by slight changes in pH.

Dose-inhibition analysis of NMDA activated currents for two α-phosphonate antagonists, D- and L-forms of LY 257883, indicated a 3-fold increase in equilibrium potency between experiments on voltage clamped mossy fiber hippocampal neurons conducted at pH 7.3 and pH 8.3. For the same pH range, the potency of the α-sulfonate analog of LY 257883 increased only 1.4-fold. Analysis of the kinetics of block of NMDA activated currents in response to rapid concentration jumps of LY 257883 revealed a 79% increase in its association rate at the higher pH but also a 14% decrease in its dissociation rate. No such changes in rate constants were observed for the α-carboxylate analog, LY 221501. The potency and concentration dependent association rate of LY 257883 is also consistent with an increase in concentration of the doubly charged antagonist species at higher pH.

**Compound** | **pH** | **K<sub>on</sub> (μM<sup>-1</sup>s<sup>-1</sup>)** | **K<sub>off</sub> (s<sup>-1</sup>)** | **K<sub>i</sub> (μM)**
--- | --- | --- | --- | ---
LY 257883 | 7.3 | 3.36 ± 0.99 | 0.85 ± 0.04 | 0.45 ± 0.05
LY 257883 | 8.2 | 5.92 ± 2.64 | 0.71 ± 0.20 | 0.14 ± 0.01
LY 221501 | 7.3 | 7.30 ± 2.58 | 3.45 ± 0.26 | 1.34 ± 0.15
LY 221501 | 7.0 | 7.60 ± 1.94 | 4.25 ± 0.37 | 0.92 ± 0.10

M.B. is supported by the National Research Council.

612.3 EFFECTS OF EXOCYGENOUS GLUTAMINE (GLN) ON CA1 FIELD POTENTIALS AND SPONTANEOUS ACTIVITY IN RAT HIPPOCAMPUS IN VITRO. M. KAWAKA, J.M. KENNEDY AND J.I. KUFFNERBERG. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

GLN, normally present in brain extracellular fluid and CSF in the range of 0.2 to 0.5 mM, plays an important role in energy and neurotransmitter metabolism. However, it is not included in ACSF used for in vitro slice studies. To study the effects of GLN on the latency to onset (LAT) and amplitude (AMP) of field potentials and spontaneous activity both in normal and in 9.4 mM K<sup>+</sup> ACSF, rat slices were equilibrated for 60 min in a submerged slice chamber either with or without 0.5 mM GLN in the ACSF. The slices were monitored for 15 min and then the media switched to normal or 9.4 mM K<sup>+</sup> ACSF with or without 0.5 mM GLN. When 0 mM GLN was removed from ACSF, an immediate increase in LAT was measured in 5 of 5 slices when normal ACSF was added. Full rhythmic bursting developed in 3 of the 5 slices while small infrequent bursts occurred in 1 other slice. When 0.5 mM GLN was added along with 9.4 mM K<sup>+</sup> the number of action potentials evoked increased 710±3% and the frequency of channel events was increased two-fold. These data indicate that while 0.5 mM GLN appeared to decrease the onset and severity of K<sup>-</sup>-induced spontaneous bursting activity, the effect on the evoked population spike was slight and limited to a small attenuating effect on LAT.

612.4 ISOPROTERENOL INHIBITS NMDA-INDUCED CURRENTS IN XENOPUS OOCYTES. A. Omerovic* and S. R. Kelso. Dept. of Biological Sciences, Univ. of Illinois at Chicago, Chicago, IL 60608, USA.

The modulatory action of beta-1 adrenergic agonists on NMDA receptors was studied in Xenopus laevis oocytes injected with total rat brain mRNA. Using two electrode voltage-clamp techniques, changes in NMDA currents were measured during bath application of different concentrations of isoproterenol. After incubation in isoproterenol (100 and 1000 μM) for 1-30 minutes, the NMDA current was not significantly changed. However, in the presence of isoproterenol (200 μM), the NMDA current was significantly and reversibly reduced to 54.3 ± 3.6 % of control (n=11). The minimal inhibition (10%) was obtained with 1 μM, the maximal (96%) with 2 μM (IC<sub>50</sub> = 200 μM). The beta-1 adrenergic antagonist atenolol (100-200 μM) did not block the isoproterenol effect. A dose-response curve for NMDA in the presence of 200 μM of isoproterenol showed a noncompetitive antagonism and was maximal at 1 μM. Isoproterenol was also effective on NMDA currents for the homologous NMDA subtype of glutamate receptors. Kainate, AMPA and k-ACPD responses were not changed significantly in 5 cells tested. Isoproterenol inhibition of NMDA current was not voltage dependent. NMDA conductance was reduced and reversal potential was not changed by isoproterenol (5 cells). A similar reduction (to 71.4±2.7% of control) was seen in 6 cells perfused with saline in which Ca<sup>2+</sup> was replaced by Ba<sup>2+</sup> in order to eliminate the contribution of Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channel in NMDA current. The inhibitory effect of isoproterenol on NMDA currents was mimicked by norepinephrine; bath application of 100 μM and 1 μM of NE reversibly reduced the NMDA current to 44.1±2.9% and 13.6±0.9% of control, respectively. This inhibition was not blocked by atenolol. The results suggest direct inhibitory effect of isoproterenol on NMDA receptors in total rat brain injected oocytes. Supported by NIH grant NS34591 and Klingenstein Fund.


The glutamate receptor subtype, N-methyl-D-aspartate (NMDA), is activated by a variety of ligands. We have used the cell-attached patch-clamp technique with agonists included in the patch pipette, to study the single channel properties of the NMDA channel, with different ligands. The compounds used included the D and L forms of N-methyl-aspartate (NMA) and the D and L forms of homocysteic acid. All of the ligands activated the NMDA channel. The modulatory action of isoproterenol was selective for NMDA subtype of glutamate receptors. Kainate, AMPA and k-ACPD responses were not changed significantly in 5 cells tested. Isoproterenol inhibition of NMDA current was not voltage dependent. NMDA conductance was reduced and reversal potential was not changed by isoproterenol (5 cells). A similar reduction (to 71.4±2.7% of control) was seen in 6 cells perfused with saline in which Ca<sup>2+</sup> was replaced by Ba<sup>2+</sup> in order to eliminate the contribution of Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channel in NMDA current. The inhibitory effect of isoproterenol on NMDA currents was mimicked by norepinephrine; bath application of 100 μM and 1 μM of NE reversibly reduced the NMDA current to 44.1±2.9% and 13.6±0.9% of control, respectively. Isoproterenol inhibition was not blocked by atenolol. The results suggest direct inhibitory effect of isoproterenol on NMDA receptors in total rat brain injected oocytes. Supported by NIH grant NS34591 and Klingenstein Fund.
11.6.2


Mg²⁺ less potently blocked the NMMA-evoked depolarization of CA1 hippocampal CA1 pyramidal cells in 10-15 day old hippocampal slices and blocked ≈75% of the NMDA-evoked depolarization in CA1 pyramidal cells. In contrast, in intact hippocampal slices and hippocampal slices treated with tetrodotoxin, ≈75% of the NMDA-evoked depolarization was blocked by Mg²⁺ at 100-150 μM.

Under these conditions, we studied the effects of Mg²⁺ on the binding of [3H]TCP to hippocampal membranes. The initial rate of binding decreases with the addition of Mg²⁺ to the hippocampal membranes. The rate is influenced by the state of the receptor and its membrane environment, but not by membrane potential, intracellular constituents or the presence of non-selective antagonists. These data support the existence of NMDA receptor subtypes that can be distinguished pharmacologically using [3H]TCP binding. Under control conditions, 7-chloro-5,8-dihydroxy-3-propyl-1-phosphonic acid (CPP) was measured in both regions during the developmental periods. The effects of a series of increasing, bath applied antagonists were assessed using the local negative extracellular field potentials produced by iontophoretic application. Inhibition curves were produced for the two regions during each age window. These relationships were used to estimate the concentrations of DC'(AP)S needed to produce a 50% inhibition in each case. The antagonist was more potent in both regions of the immature hippocampal slices than in the older tissue.

Within each age group the potency did not seem to differ significantly between regions. The 50% inhibition concentration in immature hippocampal CA1/SR and CA3/PTZ was approximately 4 μM while the mature regions required approximately 14 μM. These differences in antagonist potency are further indications of developmental changes in the hippocampal NMDA receptor population. Supported by NIMH grant NS 16064.

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**11.6.11**

**MODULATION OF THE DEVELOPING NMDA RECEPTOR-CHANNEL COMPLEX BY PHENCYCLIDINE IN RAT BRAIN.** R. Srour, S.R. Zukin and D.L. Dow-Edwards. Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York and Department of Pharmacology, State University of NY Health Science Center, Brooklyn, New York.

The NMDA receptor complex has been shown to be involved in the activity-dependent synaptic plasticity in developing animal. The present study was directed at determining the effects of chronic postnatal phencyclidine (PCP) exposure on [3H]MK-801 binding properties. We have earlier shown that [3H]MK-801 binding can be used as a marker for NMDA channel activity. Rat pups were daily injected (i.p.) with PCP (5 mg/kg) or saline beginning on postnatal day 5 till day 15. Animals were sacrificed on postnatal day 21, six days after the last injection. [3H]MK-801 binding was measured in well-washed cortical and control forebrain CSMS, both in the virtual absence of any excitatory amino acids and under various degrees of channel activation i.e. in the presence of L-glutamate and/or glycine. In the presence of any excitatory amino acids and glycine, data from equilibrium saturation experiments indicate that PCP treatment produced a decrease in the density of high-affinity [3H]MK-801 binding sites. When binding was carried out in the presence of L-glutamate alone or in the added presence of glycine there was no difference in [3H]MK-801 binding between PCP- and saline-treated brains. The authors conclude that chronic PCP exposure during development can alter NMDA receptor regulation.

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**11.6.12**

**REGIONALLY DISTINCT NMDA RECEPTOR SUBTYPES DISTINGUISHED BY [3H]TCP BINDING TO RAT HIPPOCAMPAL SLICE.** R.J. Bredt, M.C. Martin, and J.W. Iversen. Dept. of Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The existence of NMDA receptor subtypes has been proposed, but has yet to be verified with detailed pharmacological analysis. We performed in vivo quantitative [3H]TCP autoradiography in rat brain, under baseline and maximally stimulated conditions, to test potential NMDA receptor subtypes. Displacements of [3H]MK-801 binding were compared with the NMDA antagonists (D-carbamoylphenylalanine-4-ylpropyl-l-phosphonic acid (CPP) and the strychnine-sensitive glycine site antagonist, 7-chlorokynurenate (7-ClKyn)).

Under control conditions, several brain areas were differentially sensitive to CPP displacement of [3H]MK-801 binding. CPP inhibited [3H]MK-801 binding in outer cortex (OC) and inner cortex (IC) with Ki values of 3.2-6.8 μM, while in medial striatum (MS), lateral striatum (LS) and CA1 and dentate gyrus (DG) of hippocampal Ki values were 1.1-1.5 μM. In medial thalamus (MT) and lateral thalamus (LT) the Ki was 0.7-1.0 μM (approximately 1.0-2.0 μM, Fisher P≤0.05). In the presence of glycine (3 μM), the Ki values between regions remained a similar relationship with the exception of the Ki for OC which increased 2.5-3.0 times more than other regions. 7-ClKyn inhibited [3H]MK-801 binding also distinguished regionally distinct subtypes which differed from CPP displacements. Under control conditions, 7-ClKyn inhibited [3H]MK-801 binding in OC, IC, MS and LS with Ki values of 6.3-8.6 μM, while in CA1, DG, LT and MT, Ki values were 11.4-13.6 μM (the groups differed significantly, P < 0.001, Fisher P≤0.05). In the presence of glycine (3 μM) the Ki values shifted similarly, with the exception of OC which increased 1.5 times more than other areas.

These data support the existence of NMDA receptor subtypes that can be distinguished pharmacologically using [3H]TCP binding. By analogy to the GABA A receptor, one would predict the existence of regional differences in subunit composition of the NMDA receptor. Supported by USPHS NS19613, AG08671.
612.13

The N-methyl-D-aspartate (NMDA)-evoked current recorded at early stages of development from hippocampal neurons kept in culture was very sensitive to Pb2+ but, after the second week of culture the sensitivity to this cation decreased significantly. The technique of recording in the standard patch- clamp technique. NMDA (5-100 μM) and glycine (0.5-10 μM) were delivered to the neuron under whole-cell clamp condition via a U-tube loaded 50-100 μm away. Pb2+ at concentrations of 1 to 100 μM was temperature and extracellular perfusion medium. The perfusion medium contained (mM), NaCl 165, KCl 1.5, CaCl2 2.0, HEPES 5, MgCl2 10, and pH 7.3 (340 mOsm). The micropipette solution contained (mM), CsCl 80, CsF 80, CsEGTA 10 and HEPES 10 (pH 7.3, 330 mM). The patch micropipette resistance was 2.4 MΩ. The hippocampal culture was obtained from 1-day-old rat pups and the cells were used 3-4 days after plating. Pb2+-induced inhibition of NMDA-current was concentration-dependent at the range over 1-10 μM, although this effect was reduced at higher doses. The inhibition was most prominent during the first week of cell culture, and reduced along with maturation. The current/voltage relationship disclosed a voltage-independent inhibition of NMDA current obtained from 1-day-old rat pups and the cells were used 3-4 days after plating. Pb2+-induced inhibition of NMDA-current was concentration-dependent at the range and Pb2+-induced inhibition demonstrated that Pb2+ was a non-competitive antagonist of the NMDA-site of the receptor-channel complex. The same analysis for the glycine-site showed a non-linear relationship between glycine concentration and Pb2+-induced inhibition, and suggested heterogeneity of glycine-sites. The present results show 1) the NMDA receptor may undergo some molecular conformational adjustment during maturation, and as a result, Pb2+ sensitivity is markedly decreased. 2) the lowest apparent Vm of the electrical field of the channel, and 3) there may be at least two types of glycine-site which differ from each other in their sensitivity to Pb2+. (Supported by U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88-C-8119)

612.15

The effect of N-methyl-D-aspartate (NMDA) on the intracellular free calcium concentration (Ca2+) was determined in hippocampal neurons in cell culture (a cell in tissue bath preparation) isolated from mouse hippocampus loaded with the fluorescent indicator, Indo 1. NMDA increased Ca2+ in hippocampal neurons with an EC50 of 30 μM. Ethanol inhibited NMDA-stimulated Ca2+ increases in a noncompetitive manner with an EC50 of 67 mM. NMDA responses were markedly temperature-sensitive, but lowered temperature (from 32 to 22°C) did not alter the relative degree of ethanol inhibition. Preincubation with ethanol altered the EC50 of ethanol induced inhibition. However, ethanol inhibition was reduced, in a concentration-dependent manner, by the glycine and Mg-activated current (Ca2+), Mg clearance from the extracellular space. As shown previously, the NMDA-activated current in these neurons is sensitive to the glycine or proton modulatory site and the NMDA receptor channel complex may be regulated by the glycine or proton site. These results show that ethanol inhibits the NMDA-mediated increase in intracellular calcium, and suggest that this effect is mediated by the glycine or proton site of the NMDA receptor channel complex.

612.17

The kinetics of the NMDA receptor channel complex have yet to be fully characterized. While the kinetics of many non-zero conductance states that have been observed, experimentally, are well understood, the kinetics of electrostatically invisible zero conductance states is much more difficult. Kinetic parameters of zero conductance states may be estimated from the use of computer simulations.

A kinetic model of the NMDA receptor channel complex is proposed which includes, glycine, Mg, and mGluR1 at independent sites. The model assumes the existence of three distinct channel states: open, closed and desensitized. Kinetic parameters which include binding constants and state transition rates were fitted. The model was fitted to experimental data. Thus, kinetic parameters which are presently experimentally inaccessible could be described. The model fits experimental data well and provides a framework for describing the kinetics of the NMDA receptor channel complex.

612.18
CONTRIBUTIONS OF NMDA AND NON-NMDA RECEPTORS TO VISUALLY EVOKED POTENTIALS IN ALBINO RATS. D.F. Sisson, J. Siegel, B. Cox and J. Grant. School of Life and Health Sciences and Department of Psychology, University of Delaware, Newark, DE 19716.

Excitatory amino acids (EAA) are the putative transmitter of neurons that project from LGN to visual cortex (OCl). Because early components of visually evoked potentials (VEPs) are indicatives of geniculo-cortical activity, the effects of 2-amino-5-phosphonopentanoic acid (APV), a non-NMDA receptor antagonist, and 6-cyano-7-nitroquinolinolaximine-2,3-dione (CNQX), a non-NMDA receptor antagonist, on flash VEPs from albino rats anesthetized with chloral hydrate were evaluated.

Drugs were superfused over left OCl using a cortical cup. VEPs were recorded from this cortical surface and from the unoperated, right, cortex simultaneously.

CNQX decreased amplitudes of VEP components N7 and N10 in a dose-dependent manner; neither N7 nor N10 were affected. This effect was reversed after 30 min of CSF wash. APs at concentrations up to 200 μM had no effect on VEP amplitude. CNQX has an effect similar to kynurenic acid, a non-specific, EAA antagonist. These results suggest that non-NMDA receptors generate epp's in response to visually driven input into primary visual cortex. These epp's are reflected in N7. P1 is either presynaptic or reflects a fast, non-EAA, visual pathway.

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**613.1**

**ETHANOL INHIBITION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR FUNCTION: SELECTIVE INTERACTIONS WITH THE GLYCINE RECOGNITION SITE.**

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Ethanol inhibits N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission. To elucidate the mechanism of this inhibition, we examined effects of ethanol on the binding of ligands to the NMDA and glycine recognition sites of the NMDA receptor (N-[2-hydroxy-5-methyl-4-isoxazolepropionic acid] ([H]TCP) and glycine, respectively). Ethanol inhibited N-[2-hydroxy-5-methyl-4-isoxazolepropionic acid] ([H]TCP) binding by a bimolecular inhibition of glycine recognition site and inhibited glycine-dependent binding of [H]TCP. By contrast ethanol had no effect on NMDA-sensitive [H]TCP-glutamate binding or on the agonist-dependent binding of an NMDA channel blocker (CNQX). Ethanol inhibited [H]TCP binding to a strychnine-insensitive glycine recognition site and inhibited the glycine-dependent binding of [H]TCP. By contrast ethanol had no effect on NMDA-sensitive [H]TCP-glutamate binding or on the potency of glutamate stimulation of [H]TCP binding. Ethanol reduced [H]TCP binding by decreasing the maximum number of detectable sites (without altering binding affinity) and similarly reduced glycine-stimulated [H]TCP binding but did not reduce glycine binding. Ethanol inhibited NMDA receptor function by modifying the glycine binding site so as to prevent ligand binding. This action of ethanol is similar to that of zinc. Thus regulation of the glycine binding site is a common mechanism for structurally diverse allosteric regulators of the NMDA receptor.

**613.2**

**EFFECTS OF ETHANOL ON GLUTAMATE/GLYCINE ACTIVATION OF [H]TCP BINDING: A MODEL FOR THE NMDA RECEPTOR-ION CHANNEL.**

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Work from our laboratories back to 1978, has implicated brain glutamate receptors in the CNS actions of ethanol (ETOH) (e.g., Biochem. Pharm., 1978, 27:165). Recent observations in several laboratories that the NMDA subtype of glutamate receptors is unusually sensitive to ETOH at physiologically relevant concentrations led us to use a model of NMDA channel activation, i.e., glycine-stimulated activation of [H]TCP binding, to explore the site on the channel complex with which ETOH interacts in brain membranes. When both glutamate and glycine were present at 100 μM, ETOH inhibited [H]TCP binding marginally (~ 15%) at concentrations up to 300 μM. When the effects of 1000 μM ETOH were examined with varying concentrations of each agonist alone, ETOH had little or no effect on glutamate stimulation of the [H]TCP binding but did inhibit activation by glycine. Dose response curves for ETOH measured at 100 μM glycine alone revealed an IC50 of ~ 5 mM ETOH and 90% inhibition of the glycine stimulated response by 10 mM ETOH. Experiments to determine whether glycine could overcome the effects of ETOH indicated that, when ETOH was present at 25 μM, its inhibitory effects could be overcome by glycine concentrations greater than 10 μM. In agreement with studies involving [3H]Ca influx, it appears that the site through which glycine activates TCP binding is likely to be the major site with which ETOH interacts. Studies are underway with the purified NMDA receptor complex reconstituted in liposomes to determine the effects of ETOH on the isolated protein complex which does exhibit pronounced glutamate/glycine activation of TCP binding. ([NIAAA grant #AA04732, DAAD-88-0017 from ARO, and the Cir. for Biomed. Res., Univ. of Kansas.]

**613.3**

**GLYCINE MEDIATED ENHANCEMENT OF AMINO-3-HYDROXY-5-METHYLSOLOXAZOLE-4-PROPIONIC ACID (AMPA) RECEPTOR FUNCTION.**


N-[2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid] ([H]AMPA) activates a high conductance non-NMDA receptor. Preincubation with glycine (2 μM) marginally (12%) increased the magnitude of [H]AMPA binding marginally (~12%) at concentrations up to 200 mM. However, an increase of 80% was observed at 2 μM, and 90% at 10 μM. This enhancement was not seen in studies involving 15 Ca influx, it appears that the site through which glycine activates TCP binding is likely to be the major site with which ETOH interacts. Studies are underway with the purified NMDA receptor complex reconstituted in liposomes to determine the effects of ETOH on the isolated protein complex which does exhibit pronounced glutamate/glycine activation of TCP binding. ([NIAAA grant #AA04732, DAAD-88-0017 from ARO, and the Cir. for Biomed. Res., Univ. of Kansas.]

**613.4**

**THE AMPA/QUISQUALIC RECEPTOR IS A SUBSTRATE FOR CALPAIN.**

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The subunit of glutamate receptors selective for AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) is the largest component of postsynaptic responses evoked at glutamatergic synapses and recent data by Staubli et al. (Proc. Natl. Acad. Sci. USA 87:3143, 1990) have identified the conductance of AMPA receptors as the variable of expression in LTP: a lasting enhancement of synaptic strength triggered by postsynaptic Ca2+ influx. It is of interest, then, to elucidate the mechanisms regulating these receptors, especially those which are activated by Ca2+. We have found the AMPA receptor to be a good substrate for the macromolecular-requiring form of the Ca2+-activated neutral proteinases (calpains). In vivo digestion of synaptic plasma membrane proteins (prepared from telecephalon) with 1 μM calpain I per mg membrane protein (in 20 mM HEPES/Tris, 35°C, 0.5 μM Ca2+ allowed 74% result in the cleavage of the AMPA receptor and a consequent loss in specific [3H]AMPA binding. As evidenced by the use of a polyclonal antibody specific for Glu-R1 (one of the subtypes of the AMPA receptor, cloned by Hollmann et al. Nature 342:643, 1989), this cleavage is characterized by the generation of a 95 kDa intermediate from the parent 102 kDa band within 30 min, followed by a gradual and almost complete loss of immunoreactivity on immunoblots. A similar cleavage profile was observed when hippocampal slices were depolarized in the presence of NMDA. The specificity of the anti-Glu-R1 antibody was confirmed by the finding that the cleavage profile with a protein which had been photoaffinity labeled with [3H]CNQX (an AMPA receptor antagonist), i.e., a loss of labeling at 102 kDa on immunoblots when the antibody was preabsorbed with the immunogen, and ii) comparable increase in specific [3H]AMPA binding and immunoreactivity following successive chromatographic steps. Determining whether this cleavage represents a way to change receptor properties or merely a mechanism of turnover will require functional studies of partially cleaved AMPA receptors. (Supported by AFOSR #89-0383.)
613.5

SUBSTANCE P AND POLYAMINES MODULATE THE NMDA RECEPTOR OPERATED ION CHANNEL BY SIMILAR MECHANISMS.


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Substance P (SP) has recently been shown to enhance NMDA receptor elicited responses in spinal neuron preparations. We hypothesized that SP and the polypeptides spermine and spermidine, potentiate glutamatergic responses by a similar mechanism. SP 1-14, 1-7 and C-terminal SP fragments 7-11, 8-11 and 9-11 were tested for possible interaction with the NMDA receptor operated ion channel. SP 8-11 was the most efficacious fragment. Conventional (60-200 μM), increasing [3H]MK801 binding 900% in well washed membranes and 20% in the presence of maximal glutamate and glycine concentrations, 100 and 10 μM respectively. SP fragments did not have affinity for or affect the kinetics of either [3H]Glycine or [3H]CGS19755 binding. The enhancement of [3H]MK801 binding was eliminated by glutamate or glycine receptor antagonists as well as putative polyaniline antagonists putrescine, cadaverine and argine. SP 8-11 increased the association rate of [3H]MK801 without affecting dissociation or number of binding sites. This is consistent with enhanced channel accessibility to [3H]MK801. These results suggest that SP fragments modulate the NMDA operated channel by a mechanism similar to polyanines.

613.7

CHRONIC NEONATAL MK-801 TREATMENT RESULTS IN LONG-LASTING CHANGES IN NMDA RECEPTOR SENSITIVITY AND INCREASED SEIZURE SUSCEPTIBILITY.


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We previously reported that there may exist two Mg2+ sites on the N-methyl-D-aspartate (NMDA) receptor ion channel complex; the high affinity Mg2+ (±300 μM), which stimulates and low affinity Mg2+ (<1 μM), site, which inhibits. We also showed that Gpp(NH)p decreased the affinity of Mg2+-stimulated [3H]TCP binding. In the present study, we investigated a possible interaction of the GTP binding site and high affinity Mg2+ site or glycine site. The binding of [3H]TCP to rat cerebral corticos was determined as described before (Neurosci Lett 119; 9-11, 1990). [3H]TCP, GTPyS, Gpp(NH)p and GDP potently inhibited Mg2+-stimulated [3H]TCP binding (by more than 50 %), whereas ATP, CTP, GMP, cGMP, TTP and guanosine exhibited no significant inhibition. Gpp(NH)p reduced Mg2+-stimulated [3H]TCP binding by only 16 % in cholora toxin (CTX)-, but pertussis toxin (Ptxx),- treated membranes. On the other hand, Omp(NH)p inhibited the glycine-stimulated [3H]TCP binding to CTX- and Ptxx-pretreated membranes by 31 % and 29 %, respectively. The present findings indicate that the high affinity Mg2+ site may be coupled to CTX sensitive G-protein (Gs) and glycine site may be coupled to both CTX and Ptxx sensitive G-protein (Gs and Go).

613.9

DIFFERENTIAL EFFECT OF SUBCHRONIC COMPETITIVE AND NON COMPETITIVE NMDA RECEPTOR ANTAGONISTS ON HABITUATION IN RATS. M.P. Pellicano*, A.Cerbone and A.C. Sadile.

SPON: European Brain Research, Neuborgweg 33, 1105 AZ Amsterdam, The Netherlands.

Persistential effects of neonatal interference with NMDA receptors were investigated by measuring 1) NMDA receptor sensitivity to its agonist and antagonista and 2) susceptibility to "kindling" evoked seizures. Animals were neonatally treated with MK-801 (two s.c. injections per day, 0.25mg/kg, on postnatal days 2-18) and micro-iontophoretically tested for NMDA receptor sensitivity in the hippocampal CA1 pyramidial cells at PND 70-100. In a dose-dependent fashion MK801 evoked responses were more severely suppressed by APV, but not by non-competitive NMDA receptor antagonists, or drug vehicle intraperitoneally twice a day for 7 days. They were all tested in a Lat-maze 12 hr after the last injection. Additional injections were given 12, 24 and 36 hr after testing. Retention was run 48 hr later. Habituation of activity and defecation was measured by the between-test decrement in corner-crossings and rearings (with prevailing cognitive and noncognitive meaning, respectively) and of fecal boil (non cognitive). Subchronic blockade of NMDA receptors by low doses of competitive antagonists had no effect on habituation of horizontal and vertical activity. In contrast, non competitive antagonists impaired habituation of horizontal and vertical activity during the entire test, but only MK-801 did it significantly (P < 0.025). Thus, atoretic and isorotic blockade of high-affinity NMDA receptors differentially affect the processing of cognitive and noncognitive behavioral components, as suggested earlier (Behav Brain Res., 28:187, 1990). (Supported by CNR/MURST 40% grants).

613.6

EFFECTS OF STRESS AND CORTICOSTERONE ON THE BINDING PROPERTIES OF GLUTAMATE RECEPTORS.

G. Toce, T.J. Shors, S. Standley*, M. Baudry, R.F. Thompson.


The hippocampus is particularly vulnerable to the deleterious consequences of both acute and chronic stress. Since glutamate is the main excitatory neurotransmitter in the hippocampus, we investigated whether either the NMDA or AMPA subtypes of glutamate hippocampal receptors were affected in response to stress. Corticosteroid administration by quantitative ligand binding autoradiography using [3H]-TCP and [3H]-AMPA as ligands for the NMDA and AMPA receptors, respectively.

Male Long-Evans rats were exposed for 1 hour to restraint plus 60, 1 mA, and 1 sec tailshocks. Naive controls received no handling at all and another group was injected with corticosterone (1mg s.c.). While no change was observed in the binding properties of the [3H]-TCP under any condition, [3H]-AMPA binding was significantly decreased in several areas of the hippocampus of corticosterone-injected rats relative to naive controls. Restraint plus tailshock, however increased AMPA binding, even though corticosteroid levels were high. These results indicate that glucocorticoids may down regulate the AMPA receptor, but that some forms of stress trigger additional mechanisms that override the effects of glucocorticoids.

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613.8

CHLORIDE TRANSPORT BLOCKERS PREVENT NMDA-PCP RECEPTOR CHANNEL COMPLEX ACTIVATION.


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Activation of the N-methyl-D-aspartate (NMDA) receptor-channel complex has been implicated in a number of physiological and pathological phenomena. In particular, it modulates the excitotoxic effect of the neurotransmitter glutamate. The NMDA complex contains several distinct binding sites targets for regulation by endogenous as well as exogenous compounds. In cultured spinal cord neurons we found that blockers of chloride transport (furroesimide, a widely used loop diuretic, and the related compounds piretanide and bumetamide; niflumic and fluminamides, used as antiinflammatory agents) prevented NMDA receptor activation. The inhibitory effect was dose-dependent and specific for this class of glutamate receptor, as kainate- and AMPA-activated currents were only slightly altered. Antagonism of NMDA mediated currents by chloride transport blockers was voltage-independent and showed fast on-off kinetics. The action was non-competitive with NMDA and did not arise from interaction with the Zn2+ inhibitory site since blockade of NMDA induced responses by furroesimide and Zn2+ was additive. The inhibition was greater in low concentrations of glycine but it could not be overcome by increasing glycine concentrations (up to 100 μM). In contrast the inhibition was attenuated by the polyamine spermine. Since the presence of spermine was not required for inhibition to develop, we conclude that chloride transport blockers are non-competitive antagonists of the NMDA receptor acting as inverse agonists of the polyamine receptor and/or as agonists of the postulated inhibitory polyamine site. This action may explain the protective effect that has been shown for some of these drugs in neuronal degeneration.
613.11


It has been previously shown that pyrazole, an alcohol dehydrogenase inhibitor, is able to interact with NMDA receptors on hippocampal neurons (Neurosci. Abstr., 16:86, 1990). In this work, using the single channel patch-clamp technique we evaluated the effects of 4-methylpyrazole on outside-out patches excised from cultured fetal rat hippocampal neurons. After the outside-out configuration was achieved, the patch was placed inside a glass mini-pipe connected to a perfusion system that was used to deliver the drugs. Nominally Mg2+-free solutions were used for the recordings. 4-methylpyrazole (10 μM) induced channel openings that resembled those activated by NMDA and by low concentrations of pyrazole. The voltage-current relationship showed that these channels had a predominant conductance value of 50 pS. Openings interrupted by numerous brief closures were seen and the burst pattern was more pronounced at hyperpolarized potentials. As the membrane was hyperpolarized, the number of events per burst increased and the duration of individual openings decreased. This channel activity was blocked by APV (100 μM), a competitive antagonist of NMDA receptors. In contrast to pyrazole, which activated and blocked the NMDA receptors at similar concentrations, the methylated derivative did not block the NMDA channels at the concentrations tested. These findings coupled to a very simple and rather rigid structures of pyrazole make this series of compounds promising probes for structure-activity relationship analysis regarding activation and blockade of the NMDA receptors. Supported: U.S. Army Mod. Res. & Devel. Comm. Contr. DAMD-17-88-C-4119, FINEP/UMAB Mol. Pharmacol. Training Program and CNPq-Brazil.

613.13

N-METHYL-D-ASPARTATE UNILATERALLY INJECTED INTO THE STRIATUM OF RATS PRODUCES CONTRALATERAL CIRCLING: ANTAGONISM BY 2-AMINO-7-PHOSPHONHEPTANOIC ACID AND CISS-PHENOXOTHIENOL. P.K.Thanos, K.Khamandas and R.J.Beninger. Department of Psychology, Queen’s University, Kingston K7K 3N6, Canada.

To evaluate the contribution of striatal glutamate receptors to motor control, circling responses were observed in rats following unilateral intrastriatal microinjections of the agonist, N-methyl-D-aspartate (NMDA) or the antagonist, 2-amino-7-phosphonoheptanoic acid (APH). The role of dopamine (DA) in NMDA-produced circling also was evaluated. In experiment 1, an NMDA dose of 5.0 μg (in 0.5 μl), but not 0.5 or 0.05 μg produced significant contraversive circling. In experiment 2, an APH dose 10.0 μg but not 1.0 or 0.1 μg produced significant contraversive circling. In experiment 3, microinjections of the ineffective 0.1 μg dose of APH or a dose (20 μg) of the DA antagonist, ciss-phenoxothi enol, that did not produce circling when administered alone, significantly reduced the circling response produced by the 5.0 μg dose of NMDA. NMDA produced contraversive circling in the same direction as that seen following similar unilateral injections of locomotion-stimulating DA agonists, the present results suggest that glutamate, acting via NMDA receptors, may modulate DA receptor-mediated contraversive circling of DA receptors in the same region of the striatum.

613.15

POSSIBLE INTERACTION BETWEEN NMDA AND DOPAMINE RECEPTORS. G. CANDOLFI and B. DALL’OLIO, Dept. of Pharmacology University of Bologna, ITALY, 40126.

The administration of the NMDA non competitive antagonists MK-801 (0.25 mg/kg i.p.) produced a long lasting increase in rat locomotor activity which is blocked by D-cycloserine (3 mg/kg i.p.) or spermidine (250 μg/rat). Although these results strongly suggest that this effect is specific for NMDA receptor blockade, direct or indirect DA-ergic mechanisms could be involved. In well washed membranes the (3H)-MK-801 specific binding was decreased by the "in vitro" addition of either SKF 38393 or LY 171555 (100 μM). The stimulatory effect of (3H)-MK-801 binding by glutamic acid is competitively inhibited by dopamine. In an apparently non competitive manner, Acute treatments neither with indirect DA-ergic agents neither with specific D1 or D2 DA-ergic drugs failed to change (3H)-MK-801 specific binding. Repeated administrations with MK-801 (0.25 mg/kg i.p. daily for 21 days) failed to change (3H)-MK-801 specific binding, in contrast (3H)-spiropéridol binding was decreased. Consistently LY 171555 induced hyperactivity was reduced while neither (3H)-SCH 23390 binding nor SKF 38393 induced grooming behavior were affected.

613.16

PHARMACOLOGICAL EVIDENCE SUGGESTING MULTICITY OF KAINATE RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM. Atsawa H.1, Nakamura S.2, Ishida M.2 and Shinozaki H.1. 1National Institute of Neuroscience, Tokyo 187. 2The Tokyo Metropolitan Institute of Medical Science, Tokyo, 113, Japan.

Effects of various kainate analogues, including acromelic acid (ACRO) and newly synthesized kainate derivatives (4-2-hydroxyphenyl)-2-carboxy-3-pyrrolidine acid (HFPA), on [3H]kainate and [3H]AMPA binding to rat spinal cord synaptic membranes were investigated. Scatchard analysis of the saturation binding experiments revealed a single population of [3H]kainate and [3H]AMPA binding sites with Kd of 6.2 ± 0.35 and 7.6 ± 1.5 nM, respectively. The rank order of the potency to inhibit the binding of [3H]kainate was domoate > HFPA > kainate ≥ MPA > quinpirole > ACRO, but the potentization of kainate ≥ MPA > ACRO ≥ L-glutamate ≥ HFPA > domoate > kainate. ACRO, HFPA, and MPAFPA depolarize newborn rat spinal motorneurons and dorsal root C-fibres more potently than kainate. Therefore, ACRO may exert the potent depolarization not through activating either kainate or AMPA receptors but through other receptors. Present results lend support to the presence of multiple kainate receptor subtypes which we have postulated from the complex systemic neurotoxic effects of ACRO and kainate in the rat.
614.1

SELECTIVE KAPPA ACTIVITY OF DYNORPHIN Ia (DYN Ia) IN MICE. V.K. Shukla*, M. Barsinait* and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada and Department of Anesthesiology, NYU Medical Center, New York, USA.

Effect of Dyn Ia (i.c.v.) was studied on the visceral pain (acetic acid-induced writhing), gastrointestinal transit and body temperature in mice. In control groups, Dyn-Ia dose dependently protected against acetic acid-induced writhing with an ED₅₀ of 1.78 nmol/mouse (95% confidence limits (CL) 0.72-0.84 nmol/mouse). Non-biphalinopholine pretreatment (10 nmol/mouse 15 min before) increased the ED₅₀ of Dyn Ia to 4.61 nmol/mouse (95% CL, 3.67-5.61 nmol/mouse). The potency ratio between control and morphine pretreated groups was 0.39 (95% CL, 0.27-0.55, P < 0.05). The ED₅₀ of Dyn Ia was 2.45 nmol/mouse (95% CL 1.82-3.31 nmol/mouse) in metaphet pre-treated (10 nmol/mouse 24 hr before) group. The potency ratio between control and methaphet pretreated group was not significant. Dyn Ia (i.c.v., 1-10 nmol) did not have any effect on gastrointestinal transit and body temperature. These results indicate that the potent antiinflammatory effect of suprasynaptic Dyn Ia in the writhing test is mediated by the stimulation of the κ opioid receptor and is not accompanied by any change in gut motility and body temperature. Supported by the MRC.

614.3


Injection of amniotic fluid (AF) or placenta has been shown to rats to enhance opioid-mediated analgesia, but not to affect opioid-mediated hyperthermia. The present study assessed the effects of injection of AF on another opioid-mediated behavior: contralateral circling induced by unilateral injection (i.t.) of morphine (i.e., VTA microinjection of morphine). We decided to test this opioid-induced behavior because its neural mechanism has been dissociated from that producing analgesia.

Rats were given unilateral morphine injections (0.0, 0.1, 0.3, or 0.4 μg into the right VTA) or un.injected. Monkeys were divided into two groups: AF or control fluid (boeuf, BIB). Circling was monitored for 1 hr following injection. The dependent measure was not random counts of contralateral circles per hour (INCC/hr). Pain threshold was assessed by tail-flick latency at 1 hr post-treatment to confirm that morphine-induced analgesia had not been produced. Each rat was tested at all doses of morphine in a balanced design.

As expected, NCC/hr among AF-injected rats (n = 4) was significantly elevated at all doses of morphine (NCC/hr = 35-60 after morphine injections; NCC/hr = 0 after vehicle injection). In contrast, INCC/hr among AF-injected rats (n = 4) was not elevated after any dose of morphine (p < 0.01) (INCC/hr = 0 after all morphine injections and after the vehicle injection). Pain threshold was unaffected by any treatment (no analgesia was produced). These results demonstrate that the effect of AF injection on opioid-mediated behavior is not limited to analgesia and is not mediated through a single neurotransmitter site.

614.4

VENTRAL TEGMENTAL AREA (VTA) AND NUCLEUS ACCUMBENS (NAS) K- OPIOID AGONIST, U50488H, INHIBITS MALE SEXUAL BEHAVIOR. M. Leyton and J. Stewart. Center for Studies in Biobehavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Québec, Canada H3G 1M8.

We previously reported that systemic injections of the κ-agonist U50488H (U50) naloxone reversely decrease both appetitive and consummatory aspects of male sexual behavior (Leyton & Stewart, 1991a,b). Moreover, selective aspects of these U50-induced deficits could be differentially reversed by infusions of the κ-agonist norbinaltorphine into the VTA, NAS and medial preoptic area (mPOA). In the present study, male rats with bilateral VTA, NAS or mPOA cannulizations were centrally administered U50 (0.005-5.00 μg/rat). Infusions of U50 into the VTA decreased the proportion of males to ejaculate, and increased the latencies to mount, intromit and ejaculate at the highest dose. Intra-NAS U50 decreased the proportion of males to intromit and ejaculate, and, the mean number of ejaculations as well as increasing the latencies to mount, intromit and ejaculate except at the highest dose. Intra-mPOA U50 did not significantly alter any measures. Comparing these findings to our previous reports suggests that mPOA-κ-receptor activation only weakly inhibits sexual behavior in otherwise unhampered males, but, synergistically potentiates the inhibition of appetitive aspects of sexual behavior induced by VTA or NAS κ-receptor stimulation. Conversely, κ-receptor stimulation in either the VTA or NAS is sufficient to delay the initiation of male sexual behavior.

614.5

NEURAL SUBSTRATES UNDERLYING AMYGDALOID CONTROL OF DEFENSIVE RAGE EJECTED FROM THE PERIAQUEDUCTAL GRAY IN THE CAT. M.B. Shukla and A. Siegel. Department of Neurosciences, N.J. Medical School, Newark, NJ 07103.

Recently, we have shown that opioid peptides powerfully suppress feline defensive rage (DR) from the midbrain periaqueductal gray (PAG) and that a major source of input to this site is the central amygdaloid nucleus (CE). This study provides anatomic/immunocytochemical evidence in support of this hypothesis. Cannula electrodes were implanted into the PAG for elicitation of DR as well as for infusion of non-selective and selective opioid antagonists and the retrograde tracer, fluorogold (FG). Opioid receptors were identified by their opioid antagonists. Monopolar electrodes were implanted into amygadaloid sites from which suppression of DR was obtained. Brain tissue was processed for autoradiography with [³H]naloxone or [³H]nalbuphine analgesia. Monax488H (U50) naloxone reversely decrease both appetitive and consummatory aspects of male sexual behavior (Leyton & Stewart, 1991a,b). Moreover, selective aspects of these U50-induced deficits could be differentially reversed by infusions of the κ-agonist norbinaltorphine into the VTA, NAS and medial preoptic area (mPOA). In the present study, male rats with bilateral VTA, NAS or mPOA cannulizations were centrally administered U50 (0.005-5.00 μg/rat). Infusions of U50 into the VTA decreased the proportion of males to ejaculate, and increased the latencies to mount, intromit and ejaculate at the highest dose. Intra-NAS U50 decreased the proportion of males to intromit and ejaculate, and, the mean number of ejaculations as well as increasing the latencies to mount, intromit and ejaculate except at the highest dose. Intra-mPOA U50 did not significantly alter any measures. Comparing these findings to our previous reports suggests that mPOA-κ-receptor activation only weakly inhibits sexual behavior in otherwise unhampered males, but, synergistically potentiates the inhibition of appetitive aspects of sexual behavior induced by VTA or NAS κ-receptor stimulation. Conversely, κ-receptor stimulation in either the VTA or NAS is sufficient to delay the initiation of male sexual behavior.

614.6

ENHANCEMENT OF MET-ENKEPHALIN STAINING IN THE RAT BRAINSTEM AS A RESULT OF SOCIAL STRESS. C.A. Cohen*, B.M. Kream*, L.E. Marchand and K.A. Nickery. Department of Psychology, Tufts University Research Building, 490 Boston Ave., Medford, MA 02155 and the Department of Anesthesiology, Tufts University School of Medicine, 136 Harrison Ave., Boston MA 02111.

Immunohistochemistry was used to test the effects of social stress on the endogenous opiate system. Results have already indicated that socially stressed animals show marked potentiation of the anabolic effect of morphine, as demonstrated by a shift in the dose response curve to the left, during the social stress experience. Within 24 hours after social confinement, there is also evidence for mild tolerance to opiate analgesia, and tolerance continues to develop as a function of time. In the current experiment an intruder male rat was placed into the home cage of a resident and subsequently exposed to attack and threat. Opiates are known to induce unambiguous signs of submission (i.e. through ultrasounds and supine postures), the intruder was placed into a protective cage for one hour while being exposed to the threat of an attack. No physical harm could come to the intruder at this time. The intruders were perfused with saline followed by 4% paraformaldehyde, at various time intervals after the social stress experience. The brains were removed and placed in fixative for two hours followed by a solution of 20% sucrose and 0.1M phosphate buffer where they remained until sectioning. The sections were then mounted in the same room and were located in proximity to the defeated animals. They were perfused and their hands were handled in the same manner as mentioned above. All brains were sectioned (30 μm) and prepared for immunohistochemistry with antibodies for met-enkephalin. Preliminary observations suggest that at the mesencephalic and pontine levels there was an increase in met-enkephalin immunoreactive elements in the central gray surrounding the aqueduct in the socially stressed animals three hours after the encounter, in comparison to controls. These changes are being monitored via radioimmunoassay and molecular biological techniques.
1540

OPIOIDS: BEHAVIOR II

FRIDAY AM

164.7

DIFFERENTIAL EFFECTS ON MOUSE LOCOCOMOTORY ACTIVITY OF MU
SELECTIVE VS DELTA-SELECTIVE OPiODS. M. E. Neuringer and S. A.
Nash. Dep. of CNS Diseases Research, Seattle, Skokie, IL 60077.

It has been suggested that mu-selective and delta-selective opioids
produce different profiles of locomotor activity after i.c.v. administration to mice.
Mickley, et al. [Brain Research 1990] 511:93-100) observed that
while mu-selective opioids such as morphine and [D-Ala2-Me Phe4-Val7]
ekephalin (DAMGO) produce large increases in horizontal movements and
decrease in vertical movements, the delta-selective opioid ([D-Pen2,5-Phe
7]enkephalin (DPDPE) increases horizontal movements to a lesser extent
and either increases (Michael-Titus, et al.) or does not affect (Mickley, et al.)
vertical movements. The current study was conducted to determine
whether the profile of locomotor activity reported for DPDPE would
extend to other delta-selective peptides. The locomotor activity of male CD-
1 mice was monitored using a device of infrared protocol-based activity monitors
(Omitech Electronics). Mice were habituated to the monitors for 20 min
prior to i.c.v. drug administration. The results show that [D-Ala2]Deltorphin I
produced a locomotor profile similar to that of DPDPE, while [D-Sar7]-
ekephalin (DSLET), [D-Thr4]-Leu-enkephalin (DTLET), and [D-Ala2-D-
Leu5]enkephalin (DADLE) produced profiles similar to the mu-selective
agents. The results are discussed in terms of delta-receptor selectivity.

164.9

MU OPIOID ACTIVITY IN MEDIAL THALAMUS: EFFECTS ON BEHAVIOR
AND DOPAMINE UTILIZATION. K.D. Carr, S. Usaj, J.W. Schweitzer* and
A.J. Ehrnfredh. Millhauzer Labs, NYU Medical Center, NY, NY 10016.

Subcutaneous injection of the mu-selective opioid agonist, DAMGO, infused into
the lateral segment of the thalamic dorsomedial nucleus, elevate stimulus thresholds
for pain vocalization and brain stimulation reward (Carr and Bak, 1988). Higher doses
produce naloxone-reversible catalepsy. In the present study, bilateral medial
thalamic (MT) infusions of DAMGO (1.0 μg) increased latencies to shuttle-escape
from gridlock, while the kappa-selective agonist, U50,488 (25.0 μg) and the
delta-selective agonist, DPDPE (10.0 μg) had no effect. Pre-infusion of the
mu-selective antagonist, CT8 (5.0 μg), blocked the DAMGO-induced increase in escape
latency while pre-infusion of the delta-selective antagonist, ICI 174,864 (5.0 μg),
did not. Local DAMGO (0.1 to 0.25 μg) reduced gridlock-induced aversion without
affecting escape from gridlock. Many of the above mentioned behavioral effects of MT DAMGO are also produced by systematically administered
neuroleptics. Moreover, MT has strong anatomical and functional connections
with several forebrain dopamine (DA) terminal regions. To investigate whether modulation of dopaminergic activity may account for the behavioral effects of MT DAMGO infusions, we used HPLC to measure DA and DBA dopamine levels in striatum, nucleus accumbens, and medial prefrontal cortex (mPFC) in rats sacrificed
30 minutes after MT DAMGO (1.0 μg) infusion. MT DAMGO elevated DopAC levels in mPFC (t (10) = 3.98, p < 0.05) and DAMGO = 0.54 ± 0.02ng/mg protein; DAMGO = 0.54 ± 0.03
mg protein; 6) = 4.37, p < 0.05) and tended to elevate DopAC levels in striatum and accumbens as well. Systematically administered neuroleptics also increase DA utilization and elevate DA metabolite levels but do so as a compensatory response to postsynaptic receptor blockade. Whether MT DAMGO alters postsynaptic sensitivity to DA release remains to be determined.

164.10

EVIDENCE FOR THE CONTRIBUTION OF NON-OPIOID MECHANISMS IN THE ANTIMICROBIAL PRODUCTION IN MICE.

Tramadol is a centrally acting analgesic with low affinity for opioid receptors and the ability to inhibit norepinephrine (NE) and serotonin (5-HT)
uptake as well as facilitate 5-HT release. The antinociceptive effects of tramadol are primarily produced by norepinephrine (NE) and serotonin (5-HT).
These findings suggested the contribution of potential non-opioid mechanisms was the focus of the present study. In the present study, tramadol
reduced conditioned place preference (CPP) and NK1 receptor antagonist. These results demonstrate the probable contribution of a serotoninergic component to tramadol antinociception at spinal sites. In concordant experiments we tested a small and ineffective dose of i.t. serotonin. When added to a subthreshold dose of morphine and desipramine, pronounced antinociception was observed demonstrating the potential of noradrenergic and opioid effects by a weak serotoninergic stimulus.

CATACLAMINES: LOCUS COERULEUS

615.1

AFFERENTS TO THE RAT LOCUS COERULEUS (LC) USING
CHOLTERATOXIN B SUBUNIT (CTB) AS A RETROGRADE
CNS Health, Vetter Laboratories, USA (1991); CNRS UA 1197, INSERM U217, Lyon, France; CNIC, Carnegie, Dep. Anat Cell Biol, OH.

CTB when iontophoretically applied and visualized with streptavidin-HRP
immunohistochemistry has been reported to be a highly sensitive retrograde
and anterograde tracer (Lupti et al., 1990). Using CTB, we have reinvestigated the afferent
fibers to the LC. Previous work using micropipettes containing CTB (4μm up, 1% CTB in PB 0.1M, pH 6.8) and the LC identified by its characteristic
discharge properties. Iontophoretic application (0.5μA, 5min, n=20) of CTB produced
injection sites that appeared to be restricted to the LC. The present results confirm major inputs from the nucleus paragigantocellularis and pretectal hypothalamic, and
dorsal raphe (DR) nuclei, and periaqueductal gray (PAG). In contrast, after all CTB
injections in the LC, we found numerous retrogradely labeled neurons in the lateral
nucleus of the substantia nigra, the lateral tegmental nucleus, and the lateral tegmental PAG. The present results confirm major inputs from
the LC. The present results demonstrate the probable contribution of a serotoninergic
component to tramadol antinociception at spinal sites. In concordant experiments
we tested a small and ineffective dose of i.t. serotonin. When added to a
subthreshold dose of morphine and desipramine, pronounced antinociception was observed demonstrating the potential of noradrenergic and opioid effects by a weak serotoninergic stimulus.

615.2

AUDITORY-EVOKED RESPONSES OF LOCUS COERULEUS (LC) NEURONS ARE ATTENUATED BY EXCITATORY AMINO ACID (EAA) RECEPTOR ANTAGONISTS IN THE AWAKE RAT. C. Jiang, A.J. Curtis, G. Dolez, R.J. Valentino, and G. Antonjon. Division of Behavioral Neurobiology, Dep. Mental Health Sciences, Hahnemann University, PA 19102.

LC neurons of behaving rats respond to monodispal non-noxious stimuli (Antonjon and Bloom, 1981). This study was designed to elucidate the transmitter mechanisms through which other stimuli may modulate LC neuronal activity. Responses of LC neurons were performed in unanesthetized rats 5-7 days after implantation surgery. Extracellular single- and multiple-unit activity were differentially recorded through stainless steel wires in the LC, and drug solutions (5 μl) were injected into the lateral ventricle. Activation of LC neurons by auditory stimuli (2.5 kHz, 50 mV 100 ms duration. 90 dB) was significantly attenuated by EAA antagonist receptors. These results demonstrate the probable contribution of a serotoninergic component to tramadol antinociception at spinal sites. In concordant experiments we tested a small and ineffective dose of i.t. serotonin. When added to a
subthreshold dose of morphine and desipramine, pronounced antinociception was observed demonstrating the potential of noradrenergic and opioid effects by a weak serotoninergic stimulus.
615.3 PUTATIVE GLUTAMATERGIC AFFERENTS TO THE NUCLEUS LOCUS COERULEUS FROM THE NUCLEUS PARAALTHALAMICUS: IMMUNOREACTIVITY AND TRANSMISSING.

C. Doolin and G. Aston-Jones, Division Behavioral Neurobiology, Dept.
Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102.

Recent investigations have found that the nucleus paraaltrhalamicus (PAG) in
the ventrolateral medulla provides a major excitatory input to the
nucleus locus coeruleus (LC). Moreover, pharmacological studies revealed
that this excitatory pathway, which resembles somatotopy, and visceral-evoked
activation of the LC, uses an excitatory amino acid (EAA) neurotransmitter.
The endogenous EAA used in this pathway is not known, but glutamate is a good candidate.
In the present study, we combined retrograde transport and immunohistochemical techniques to identify
the source of possible glutamatergic inputs to the LC. The retrograde tracer,
a wheat germ globulin conjugate to collagen gold particles (Owland-
spoilRl-Au), was injected into the electrophysiologically identified LC
targets of two rats. After survival for 5-7 days, the rats received colchicine
(100 μg iv) and were perfused 24 hours later. A mouse monoclonal IgM antibody
against glutamate-activated glutaminase (PAG, kindly provided by Dr. T. Kanebo,
Kyoto University, Japan) which is a major synthetic enzyme of glutamate, was used to tentatively identify
neurons. Numerous PAG-immunoreactive (ir) cell bodies were
observed in the PAG. Preliminary double labeling studies indicated that
PAG-ir neurons represent a substantial proportion of the LC-projecting
neurons in the PAG. These results provide morphological support for the
existence of an excitatory amino acid pathway, putatively, glutamatergic,
to LC from PAG.

615.4 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF LOCUS COERULEUS NEURONS IN MAUSDELY REACTIVE AND NON-REACTIVE RAT STAINS.

Univ. N. Iowa, Cedar Falls, IA, Dept. of Pharm. Sci., Coll. Pharmacy & A.H.P., Dept.
of Psychiatty, Sch. Med., Wayne State Univ, Detroit, MI 48202.

The Mouseley Reactive (MR) and Non-Reactie (NMR) inbred rat strains, have been shown to perform differently in various animal models for anxiety. Changes in noradrenergic function have been implicated in anxiety states. In the present study extracellular single-unit recording techniques were used to examine the physiology and pharmacology of noradrenergic locus coeruleus (LC) neurons in MR and NMR anesthetized rats. The baseline discharge rate of LC neurons in MR ("anxious") and NMR ("non-anxious") and Sprague-Dawley (SD; outbred control) rats was 2.6 ± 0.8 (n=6). 4.6 ± 0.8 (n=6) and 3.0 ± 0.8 (n=5) respectively. The sensitivity of somatodendritic alpha-2-adrenergic receptors to i.v. clonidine was also examined. An analysis of covariance (basal discharge rate= covariate) indicated that the dose-response (DR) curves for both Mauudsy strains were significantly (P<0.01) shifted to the right relative to SD rats and that NMR and MR DR curves were not significantly different from each other (P>0.23). Although these studies are preliminary, a clear difference in clonidine sensitivity was seen be tween Mauudsy and SD rats. A more complete analysis of the physiological and pharmacological characteristics is currently underway. (Supported by MH47181).

615.5 DECREASED TONIC DISCHARGE AND INDUCTION OF PERIODIC BURSTING OF LOCUS COERULEUS (LC) NEURONS AFTER ACUTE MORPHINE IN WAKING MONKEYS.


Substantial evidence indicates that the LC may be an important site of action for
endorphins. However, the effects of opiates on the electrical activity of LC neurons in conscious animals remain controversial, and never have been reproducible in primates. Here, the discharge activity of 11 neurons located in the LC region and tentatively identified as noradrenergic cells (2-8 ms spike, low frequency, burst-pause response to sound) was recorded before, and up to 4 hours after i.v. injections of morphine sulfate in a chair restrained Cynomolgus monkey (3 cells each with 0.3 mg/kg, 1 mg/kg or 3 mg/kg, and 2 cells with 10 mg/kg). After injection, the animal was allowed to recover in a standard cage. Both the rise and fall of LC burst firing were evident within 1 hour following morphine administration, the animal's eyes exhibited episodic slow drifts, the pupil diameter oscillated widely, and there were occasional short periods of
apnea. LC activity prior to drug administration was characterized tonically and regular, and close analysis revealed that these were small oscillations in discharge rate occurring at a frequency of about 0.04 Hz. At 3 to 7 min after morphine injection, LC neurons showed pronounced periodic bursting activity, which continued for the duration of the recording session. Such bursting also occurred with a frequency of 0.04-0.05 Hz, as detected by autocorrelation of unit discharge, and was most pronounced about 20 min after injection. Interpeak intervals increased with time after injection, resulting in an overall decrease of impulse activity. By 2 hrs following a high dose of morphine, LC neurons were nearly silent, even though the animal's eyes were fully open. Although most pronounced for higher doses of morphine, the above effects were observed for all cells tested. Supported by AFSOr grant 90-0147 and PHS grant DA 06124.

615.6 PHASIC LOCUS COERULEUS STIMULATION: EFFECTS IN THE RAT LATERAL GENICULATE NUCLEUS.

E. R. N. Holdefer and B. J. Jacoby.

Neuronal discharge in the locus coeruleus (LC) conveys to the CNS both a tonic information code related to behavioral state, and a phasic code elicited by sensory stimuli. Phasic activation of LC (0.26-0.62 mA at 30 Hz over 200 ms) in urethane-anesthetized rats increased spontaneous activity in the LGN by 9.4±0.6 spikes/s (means ± SEM) in 13 out of 15 cells (onset latency: 292±70 ms, duration <3 s). In some LGN neurons this increased discharge was transiently followed by a characteristic burst firing mode to a predominantly single spike mode. LC activation was much weaker in animals with stimulating electrodes in the LGN (2.5±2.2 spikes/s, t=0.41, P<0.05). Microiontophoresis of phenolamine (α-receptor antagonist: 5-10 nM) in the LGN partially reversed the increased firing after LC stimulation, but also decreased spike amplitude, making interpretation difficult. Increased LGN neuronal activity after LC stimulation was significantly reduced (2.9±4.4 spikes/s, t=4.47, P<.05) in animals pretreated (3-11 h) with a dose of AMPH (175-200 mg/kg, i.p.), known to deplete NE in the brain. We conclude that phasic LC activation can phasically activate LGN neurons in urethane-anesthetized animals.

615.7 MODULATION OF FOREBRAIN EEG BY THE LOCUS COERULEUS-NORADRENERGIC (LC/NR) SYSTEM:


We previously reported that peri-LC infusions of the cholinergic agonist, carbachol (CAB) enhance LC neuronal activity and blunt forebrain EEG activation in halothane-anesthetized rats. These effects were observed only when the infusion needle was placed within 500 μm of the LC. To determine whether LC-α- and β-receptors are involved in these effects, we examined the effects of the β-receptor antagonist, DL-propranolol (PRO, 200 μg ICV). Animals received a peri-LC CAB infusion (100 nl; 1 ng/ml) 15 min after an i.p. injection of PRO (3 μl). In contrast to the CAB study, PRO did not affect the CAB-induced activation of LC, PRO blocked or severely attenuated the LC-stimulated activation of neocortical EEG. Preliminary data suggest that an involvement of β2-receptors in mediating the LC-induced activation of forebrain EEG. The effects of inhibition of LC neuronal activity by peri-LC infusions of the α1-receptor antagonist, clonidine (125 nl; 1 ng/ml) were also examined. These infusions completely inhibited LC neuronal discharge activity for a 45-60 min period. Unilateral infusions typically had no obvious effects on cortical EEG activity. Bilateral inhibition of LC activity significantly increased the incidence of and amplitude of slow-wave activity. Recovery of EEG activity was closely correlated with recovery of LC activity. These results suggest the LC/NE system is a potent modulator of forebrain EEG activity.

615.8 PACEMAKER ACTIVITY OF LOCUS COERULEUS (LC) NEURONS: DEPENDENCE ON cAMP PROTEIN KINASE AS SHOWN BY WHOLE-CELL RECORDINGS IN BRAIN SLICES.

M. Altschuler and C. K. Achacostan, Deps. of Psychology and Pharmacology, Yale
University School of Medicine, New Haven, CT 06508.

Noradrenergic neurons of the rat LC are endogenous pacemakers that exhibit slow, tonic firing even in the complete absence of synaptic inputs. Conventional extracellular and intracellular recordings in LC slices from inbred rat strains, have been shown to perform differently in vivo and in vitro preparations. In contrast, we now report a time-dependent decline in LC spontaneous firing rates during whole-cell recording with low-resistance patch electrodes. A majority of the neurons tested gradually decreased spontaneous firing activity during whole-cell configuration, presumably due to wash-out of an intracellular molecule. Inclusion of PKA2 (a specific inhibitor of cAMP-dependent protein kinase in the patch-pipette accentuated the washout phenomenon: in all the cells tested there was a rapid cessation of firing accompanied by a 2.7 mV hyperpolarization, suggesting the involvement of endogenous cAMP and protein kinase in pacemaker activity. The inclusion of cAMP, 8-BrcAMP or the catalytic subunit of cAMP-dependent protein kinase (PKA2) in the patch-pipette prevented the loss of firing and dose-dependently increased LC firing rates. PKA2 blocked both 8-Br-cAMP and PKA2 induced firing in LC neurons. We conclude that endogenous cAMP drives tonic pacemaker activity in neurons via cAMP-dependent protein kinase and its phosphorylation pathway.
Both Transient and Tonic Activity Elevates C-Fos Levels in the Locus Coeruleus. R. Bitman*, S. Grant, D.A. Highfield, M. Jadavand, B. Benes Dept. Psychology and Psych. Univ. Delaware, Newark, DE 19716; and Wilm. Patterson College.

Increased neuronal activity induces the early intermediate gene product c-fos. The nucleus locus coeruleus (LC) is ideal for studying mechanisms coupling neuronal activity with c-fos induction. The LC contains a large number of noradrenergic neurons, and its projections to the cerebral cortex and other brain regions suggest a role in regulating behavior. The present study was undertaken to investigate whether the 5-HT release in the dorsal raphe nucleus (DRN) is regulated by cell body 5-HT1 autoreceptors. For this purpose a microdialysis probe was inserted in the DRN. Approximately 24 h later probes were perfused with artificial CSF. Cumulative doses of 8-OH-DPAT (10 nM-1 μM) were then perfused for every dose, the effect of a single 20-min pulse of 8-OH-DPAT (100nM) was tested. Dialyate 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were analyzed by HPLC(EC). The basal extracellular levels of 5-HT and 5-HIAA were 1.62 ± 0.3 nM and 6.8 ± 0.5 μM respectively. The most potent compounds were found to lower brain 5-HIAA levels following 8-acyl-2-dipropylaminotetralins (1, R = alkyl). The compounds were tested for 5-HT1a agonist activity in mice. The only known compounds which have limited oral activity are the 8-acetyl-2-dipropylaminotetralins (1, R = alkyl). The 8-acetyl-2-dipropylaminotetralins (1, R = Me, Et, i-Pr, t-Bu, and Ph) were found to have high affinity in vitro for the 5-HT1a receptor (Ki) and significantly reduced behavioral immobility in the forced swim test in rats. The compounds used were: WY 48,723, WY 50,324 and WY 47,846, respectively an agonist, a partial agonist and a competitive antagonist at the 5-HT1a receptor. WY 50,324 is also a 5-HT2 antagonist. The results received three subcutaneous injections of WY 47,846 (0.06-0.5 mg/kg), WY 50,324 (0.125-0.25 mg/kg) or WY 47,846 (10-20 mg/kg) after the 15-min pretreatment session. Behavioral immobility was measured during a 5-min test session, which occurred one hour after the last injection. The 5-HT1a agonist 8-OH-DPAT (0.5 mg/kg) and the tricyclic antidepressant desipramine (10 mg/kg) served as positive controls. WY 48,723 and WY 50,324 significantly reduced behavioral immobility as compared to saline-treated controls. These results were similar to those obtained with desipramine and 8-OH-DPAT. In contrast, WY 47,846 failed to alter behavioral immobility when tested alone, and antagonized the reduction in behavioral immobility produced by the 5-HT1a agonist. These results support our previous findings that compounds with agonist or partial agonist activity at the 5-HT1a receptor may possess antidepressant-like activity. Further studies are currently underway to ensure that the activity of these compounds in the forced swim test was not due to their effects on locomotor activity.
616.5 S-HYDROXYTRYPTAMINE HYPERPOLARIZATION IN CA3 HIPPOCAMPAL PYRAMIDAL CELLS MAY NOT BE 5-HT1A RECEPTOR-MEDIATED. R. Choll, M. L. Shuler, and P. M.WHITE. Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153.

Intracellular recording techniques were used to characterize SHT actions in area CA3 of the hippocampal slice. SHT-carbonylcyanoimidotryptamine elicited a hyperpolarization: Exm 1155 mV, Ec50 1025 mV and slope 1.9±0.7 and Exmax 13,713.4 mV, Ec50 0.5082 mV, slope 1.885. MDL 73,005EF, a selective 5-HT1A antagonist, perfused from a Schild plot analysis was 72 mV. The Kd of the 5-HT1A partial agonist BMY 7378 was 87 mV, but BMY also demonstrated an effect that was reversible. The hyperpolarization was obtained with 2M KCl or 2M K Methyl Sulphate electrodes and in the presence of 1μM TTX. The reversal potential was -105 mV in 3 mM extracellular KCl buffer and -85 mV in 5 mM KCl. We conclude that the 5-HT hyperpolarization is mediated by an increase in potassium conductance. The identity of the 5HT receptor mediating the hyperpolarization has not been conclusively identified; however, evidence against the 5-HTIA receptor includes: 1) The difference in the affinity of SHT and 5CT is 200 fold, 2) the Kd of spiperone is low for a 5-HT receptor, 3) the rank order of BMY and Spi­merone is reversed and 3) BMY acts as a non-competitive antagonist. Other agonists and antagonists are being tested to obtain Kd's and Kp's of agonists. USPHS grants MH49117, NS88512, KO2-MO08880 to SGB.


Brainstem raphe nuclei such as the dorsal/medial raphe, appear to be regulated in adulthood by somatodendritic autoreceptors of the 5-HT1A subtype. (Njoh and Magnuson, 1988). Previous work from our laboratory examining the ontogeny of low dose effects of the 5-HT1A agonist 8-OH-DPAT has led to the suggestion that 5-HT1A autoreceptors are functional in preweanling but not infant rat pups (Franbes, Spear, & Goodwin, submitted). To further assess this possibility, the present study examined the behavioral effects of microinjections of 8-OH-DPAT into the dorsal/medial raphe of postnatal day 17 (P17) rat pups. Local microinjection of 1.0 μg 8-OH-DPAT was observed to decrease 5-HIAA/5-HT ratios, with the higher dose inducing clear behavioral alterations, including the induction of flat body posture and an increase in sniffing. These data provide evidence that 5-HT1A somatodendritic autoreceptors in the dorsal raphe are functional during the late preweanling period (i.e. by 17 days of age). Future studies will explore the consequences of local microinjection of 8-OH-DPAT in younger rat pups in order to determine when these autoreceptors first become functional.


The regulation of serotonin (5-HT) release in the striatum by somatodendritic 5-HT1A autoreceptors was investigated by using in vivo microdialysis. 5-HT release was measured in the ventral caudate nucleus of rats maintained under chloral hydrate anesthesia. Dialysate samples collected in 20 minute intervals were analyzed by an HPLC equipped with a microbore column. The sensitivity of detection was approximately 0.5 to 5 pg/5 μl. Exogenous 5-HT1A agonists or antagonists were added to the perfusion medium.

The major portion of dialysate 5-HT content was neuronal in origin, as determined by sensitivity to blockade of sodium ion channels with 1.0 μM TTX (mean ± S.E.M: -78.7 ± 4.5 μV) and removal of calcium from the perfusion medium (-52.5 ± 11.2 μV). Systemic administration of the selective 5-HT1A receptor agonist 8-OH-DPAT reduced striatal dialysate 5-HT content dose-dependently and infused directly into dorsal raphe nucleus, the origin of most of the serotoninergic innervation of the striatum, reduced striatal dialysate 5-HT content to a similar magnitude. In contrast, infusion of 1.0 μg 8-OH-DPAT into the striatum via the probe perfusion medium, did not alter striatal dialysate 5-HT content. These observations suggest reductions in striatal 5-HT release are mediated by activation of somatodendritic 5-HT1A autoreceptors in the dorsal raphe nucleus. These findings are being conducted to evaluate the pharmacological and anatomical specificity of this effect. This work was supported by MH34622 & MH48125.

616.10 ELECTROPHYSIOLOGICAL RESPONSES OF HIPPOCAMPAL, SEPTAL AND RAPHE NEURONS TO MDL 73,005E, A POTENT AND SELECTIVE 5-HT1A RECEPTOR LIGAND. J. Currey, D. McCarthy, V. van den Hoof and M. Gilewski, Marion Merrell Dow Research Institute, Cincinnati, OH and Stanford, France.

MDL 73,005E. MDL 73,005E inhibits anxiolytic-like effects in behavioral paradigms, like other 5-HT1A ligands, its mode of action is uncertain. A preferential agonist effect on 5-HT1A receptors located on raphe cell bodies over those located postsynaptically in raphe projection areas has been proposed. The purpose of the present study was to examine this possibility by comparing the actions of MDL 73,005E on single neurons in the dorsal raphe (DR), hippocampus (HC) and lateral septum (LS).

In DR slices, MDL 73,005E (30 μmol/L) produced concentration-dependent inhibition of cell firing. Based upon IC50 values, MDL 73,005E was equipotent with buspirone (129 ± 34 vs 97 ± 8 nM; means ± S.E.M.). While the low concentration (1 μmol/L) of MDL 73,005E was ineffective in the presence of 1 μmol/L of buspirone, blockade of MDL 73,005E-induced inhibition. In contrast, in HC and LS slices, MDL 73,005E (1 - 30 μmol/L) did not change membrane potential but did antagonize hyperpolarizations induced by 5-HT. Buspirone induced a slowly developing hyperpolarization which was small in HC cells (30 μmol/L: 14 ± 0.34 mv) but significantly larger in LS neurons (30 μmol/L: -5.7 ± 2.7 mv).

These data indicate that MDL 73,005E autoreceptors on DR cell bodies. Little or no agonist activity was detected on HC or LS neurons and instead MDL 73,005E acted as an effective antagonist of 5-HT-induced hyperpolarization. Buspirone also inhibited DR firing; however, the small but significant activations of HC and LS postsynaptic 5-HT1A receptors by buspirone signify potential differences between its mode of action and that of MDL 73,005E.
616.11

The antidepressant properties of flesinoxan, a selective 5-HT1A agonist, have been investigated in a number of behavioral and neurochemical, neuropharmacological, neuronal and clinical antipsychotic action without delayed onset. Flesinoxan, like classical active antipsychotics, prolongs the period of active resistance of rats in the forced swimming test (0.2 to 1.8 mg/kg). The effects of flesinoxan occur at much lower doses and the active period of resistance lasts longer compared to the classical antipsychotics. Similar effects have been found with other 5-HT1A agonists such as 8-OH-DPAT and ipsapirone. Dose-dependent increases in immobility were observed in the 5-HT1A/5-HT2 antagonists, (2) may not be predictive of an active DA neurons: (1) differs from other antipsychotics. Previous studies of the effects of antipsychotics (e.g., clozapine), which lack extrapyramidal motor side-effects, only decreases the number of spontaneously active A10 DA neurons, with no effect on A9. Apomorphine did not reverse the effects of zatosetron, mediated by depolarization inactivation; and, (3) may be predictive of antipsychotics. Previous studies of the effects of antipsychotics and 5-HT1A agonists such as 8-OH-DPAT and ipsapirone, (1.0-17.8 mg) and indorenate (0.56-17.8 mg) in rats receiving two different electric shock intensities (0.16 and 0.32 mA). The data indicates that 5-HT1A agonists have a smaller antifunction effect than diazepam. The antifunction effect of each compound was of a greater magnitude at 0.16 mA intensity than at 0.32 mA. This study shows that different electric shock intensities, compounds produce a differential effect: the antifunction effects were more pronounced with the lower electric shock intensity than with the higher intensity. The present results suggest that changes in drug intensity can play distinct roles over the drug's effect in the conflict test.

616.12
EFFECTS OF 5-HT, agonists in the conflict test. A. Meneses and E. Hodot. (Spon: J.E. Villareal). Terapéutica Experimental, Depar tement of Toxicology, CINVESTAV-IPN, Mexico City, Mexico 14730.

Contradictory evidence exists concerning the anxiolytic effects of 5-HT1A agonists in the conflict test. In the present work a modification of the Vogel conflict model was used to assess different doses of diazepam (0.1-10.0 mg), ipsapirone (0.56-17.8 mg), Buspirone (1.0-17.8 mg) and indorenate (0.56-17.8 mg) in rats receiving two different electric shocks intensities (0.16 and 0.32 mA). The data indicate that 5-HT1A agonists have a smaller antifunction effect than diazepam. The antifunction effect of each compound was of a greater magnitude at 0.16 mA intensity than at 0.32 mA. This study shows that different electric shock intensities, compounds produce a differential effect: the antifunction effects were more pronounced with the lower electric shock intensity than with the higher intensity. The present results suggest that changes in drug intensity can play distinct roles over the drug's effect in the conflict test.

616.13
VARIATION IN CEREBRAL METABOLISM OF EXOGENOUS L-Tryptophan in BALB/c and C57BL/MICE. D. Ghosh and B.O. Andrews*. Department of Biochemistry, University of Texas Southern University, Houston, Texas 77024

L-Tryptophan, an essential amino acid, is metabolized in two major (i) kynurenine and (ii) serotonin (5-HT) pathways in mammalian cells. In the brain, the tryptophan 5-HT=5-hydroxyindole-3-acetic acid (5-HIAA) pathway is predominant, and 5-HT so synthesized serves as a crucial neurotransmitter in some discrete areas of the brain. Experiments were conducted to explore the existence of any difference in the cerebral metabolism of exogenous L-tryptophan (0.375 mg/kg body weight) in rats selected on the basis of their coat color: (a) dark-coat color (C57BL6) and (b) albino (BALB/c) lines of male mice which were 20 days of age. Each single whole mouse brain was extracted in 0.5 M perchloric acid (16.7% homogenate), HPLC/ Electrochemical detection was performed on supernatants obtained from crude homogenates centrifuged at 15,000 g for 15 minutes. Our results indicate that C57BL6 mice produce apparently higher levels of 5-HT and 5-HIAA than BALB/c mice. 5-HT = C57BL6 = 397 ± 12.5 ng/g (n=100) & BALB/C = 336 ± 6.9 ng/g (n=85); N = 7; P<0.05. 5-HIAA = C57BL6 = 792 ± 1.1 ng/g (n=100) & BALB/C = 445 ± 8.3 ng/g (n=56); N = 7; p<0.01.

Central 5-HT and its turnover regulate a number of physiological pathways in mammals, and this biogenic amine assumes added importance if the underlying cause of its tissue level is genetic. [Supported by NIH grant RR03045].

616.15
ZATOSETRON, A SELECTIVE 5-HT3 ANTAGONIST, DECREASES THE NUMBER OF SPONTANEOUSLY ACTIVE A10 DOPAMINE NEURONS. M. E. Stockton*, J. B. Stockton* and K. Rasmussen. Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46265.

5-HT3 receptor antagonists have been shown to have a modulatory effect on the mesolimbic dopaminergic system, leading to the hypothesis that 5-HT3 antagonists could have antipsychotic actions in schizophrenic patients. Previous studies have shown that chronic treatment with antipsychotics (e.g., haloperidol) decreases the number of spontaneously active A9 and A10 dopamine (DA) cells. However, chronic treatment with atypical antipsychotics (e.g., clozapine), which lack extrapyramidal motor side-effects, only decreases the number of spontaneously active A10 DA neurons. Apomorphine, a DA agonist, reverses these inhibitory effects of antipsychotics. Previous in vitro studies of the effects of 5-HT3 antagonists on the number of spontaneously active DA cells have produced divergent results. This study evaluated the effects of zatosetron, a 5-HT3 antagonist belonging to a different structural class than the classical 5-HT3 antagonists, on A9 and A10 single unit activity. Extracellular, single-unit recordings were made in chloral hydrate anesthetized rats and the number of spontaneously active A9 and A10 DA cells was counted. Both chronic (21 days) and acute administration of zatosetron resulted in a dose-related suppression of the number of spontaneously active A10 neurons, with no effect in A9. Apomorphine did not reverse the effects of zatosetron, indicating that the response may not be mediated via depolarization inactivation. These data indicate that zatosetron's antipsychotic action is mediated by an atypical antipsychotic action without delayed onset.

616.16
LACTATION-INDUCED ENHANCEMENT OF GASTRIC CONTRACTILITY TO SEROTONIN IN VITRO. S.B. Deshpande and K.S. Raoc. Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 003 (India).

Studied have shown that lactating rats exhibit enhanced absorptive and motor activity of the gastrointestinal tract. In order to explore the possible changes associated with lactation, the contractility of gastric fundus to serotonin (5-HT) was studied. The serotonin-evoked isometric contractions of rat fundal strip preparations obtained from four groups viz., lactating rats (LR), non-lactating non-pregnant rats (NLR), pregnant non-lactating rats (PR) and adult male rats (MR), were measured using a strain gauge transducer. ANOVA of variance revealed that the fundal tissues from LR are more sensitive to 5-HT than those of NLR (R = 18.3, P<0.001), while those from PR are less sensitive (R = 8.0, P<0.001). Furthermore, the preparations from MR were more sensitive to 3-HT than those of NLR (F = 6.3, P<0.001). While preliminary, these data suggest that fundal strips to 5-HT in lactating rats. (Supported by ICMR Grant No. 3/3-X(7)/89-BMS-III).
617.1

We have recently cloned RDC8 and RDC7, two orphans G protein-coupled receptors (Libert et al., Science (1989) 244:568-572). RDC8 has been identified as an adenosine A2 receptor (A2R) (Maenhaut et al., Biochem. Biophys. Res. Comm. (1990) 173:1169-1178) and we have now identified RDC7 as an A1 receptor (A1R). Using RDC8 and RDC7 we have generated several monoclonal antibodies. This confor to these cells the capacity to bind specifically either an A2 agonist ([3H]CGS15943) or an A1 agonist ([3H]BAY56-5023) and to stimulate or inhibit adenylyl cyclase in response to A2 or A1 agonists, respectively.

Using in situ hybridization (ISH), the mRNA encoding for this A2R is exclusively detected in the medium-sized neurons of the rat and dog striatum (Schiffmann et al., Brain Res. (1990) 519:333-337). The human A2R mRNA is also exclusively expressed by the medium-sized striatal neurons. A2R is homogeneously distributed in the adult striatum, whilst it exhibits a striosomal pattern during the early postnatal development. Moreover, an ISH colocalization study revealed that in the rat caudate-putamen A2R is mainly expressed by the GABABergen-enchekalinergic subpopulations as compared to the substance P or the cholinergic neurons. The dopamine D2 receptor is also selectively expressed by these neurons and, conversely to the A2R, inhibits cAMP formation.

617.2
MOLECULAR CLONING AND CHARACTERIZATION OF A RAT A1 ADENOSINE RECEPTOR THAT IS WIDELY EXPRESSED IN BRAIN AND SPINAL CORD. D.R. Weaver, S.A. Rixives, J.H. Siebel*, E. M. Alley*, S.M. Reppert. Laboratory of Developmental Chronobiology, Children's Service, Massachusetts General Hospital, Boston, MA 02114.

Libert et al. (Science (1984) 224:569) reported the cloning of four unidentified receptors belonging to the superfamily of G protein-coupled receptors. Two of these receptors, RDC7 and RDC8, appear to comprise a receptor subfamily. With the identification of RDC8 as an A2-adenosine receptor (BBRC 173:1169, 1990; see also abstract by J.S. Fink et al.), it seemed likely that RDC7 encodes an adenosine receptor subtype. We used the polymerase chain reaction to clone a fragment of the rat homolog of RDC7, leading to isolation of a full-length clone from rat brain. The rat cDNA encodes a protein of 327 amino acids which shares several structural features with the A1-adenosine receptor. Expression studies in COS-6 cells demonstrated that the cloned cDNA encodes a protein with high affinity for the A1-adenosine specific ligand [3H]CCPA (Kd = 88 ± 139 PM, n = 3) and appropriate pharmacological specificity (rank order for inhibiting [3H]CCPA binding: CPA > R-PIA > NECA > S-PIA). In situ hybridization studies revealed a widespread distribution of the receptor mRNA, with high levels in cerebral cortex, hippocampus, cerebellum, thalamus, brainstem and spinal cord. The distribution of the receptor mRNA is generally in good agreement with the distribution of A1-adenosine receptor/mRNA mismatches exist. The cloning and identification of A1- and A2-adenosine receptor cDNAs represents an important advance toward defining the role of adenosine receptor subtypes in CNS function.

617.3

We have utilized the polymerase chain reaction technique to selectively amplify G protein-coupled receptor cDNA sequences from rat striatal mRNA. A novel cDNA fragment was identified which exhibits considerable homology to various members of the G protein-coupled receptor family. This fragment was used to isolate a full-length rat A1-adenosine receptor cDNA from a rat striatal library. A 2.2 kb clone was isolated encoding a protein of 326 amino acids with seven transmembrane domains as predicted by hydrophy analysis. Saturable and specific binding sites for the A1 adenosine agonist, [3H]DPCPX, were identified on membranes from transfected cells. The rank order of potency and affinities of various adenosine agonists and antagonist ligands confirmed the identity of this cDNA clone as an A1 adenosine receptor. The high affinity binding of A1 adenosine agonists was shown to be sensitive to the non-hydrolysable GTP analogue, Gpp(NH)p. In adenylyl cyclase assays, adenosine agonists inhibited forskolin-stimulated cAMP production by greater than 50% in a pharmacologically specific fashion. Northern blot and in situ hybridization analyses of receptor mRNA in brain tissues revealed two transcripts of 5.6 kb and 3.1 kb, both of which were abundant in cortex, cerebellum, hippocampus, and thalamus with lower levels in olfactory bulb, striatum, mesencephalon and retina. These regional distribution data are in good agreement with previous receptor autoradiographic studies involving the A1 adenosine receptor. We conclude that we have cloned a cDNA encoding an A1 adenosine receptor linked to the inhibition of adenyl cyclase activity.

617.4
THE RAT A2 ADENOSINE RECEPTOR: MOLECULAR CLONING, EXPRESSION AND LOCALIZATION IN BASAL GANGLIA. J.S. Fink, D.R. Weaver, S.A. Rixives, B. Patenhoef*, E.M. Alley* and S.M. Reppert. Molecular Neurobiology and Developmental Chronobiology Labs, Massachusetts General Hospital, Boston, MA 02114.

Selective ligand binding and receptor autoradiography have demonstrated that the A2 subtype of adenosine receptor is expressed exclusively in striatum, n. accumbens and olfactory tubercle in rat (Eur J Pharm 1989; 184:243). Using the technique of PCR cloning with homologous sequences we have isolated the cDNA encoding the rat A2 adenosine receptor. With degenerate oligonucleotide primers corresponding to conserved transmembrane domains of G protein-linked receptors and first strand cDNA from rat brain as template, 230 bp fragments of G protein-linked receptor cDNAs were amplified using PCR. One of these receptor fragments hybridized to mRNA in rat striatum. Using this receptor fragment as probe, a full-length cDNA was isolated from a g10 rat striatal library. This receptor was highly similar to the canine G protein-linked receptor RCDC (Science (1984) 244:569) which has recently been shown to be a canine A2 adenosine receptor (BBRC 173:1169). Identification of the rat cDNA as an A2 receptor was achieved by expression in COS cells. In situ hybridization demonstrated abundant and exclusive expression of the rat A2 receptor mRNA in striatum, n. accumbens and olfactory tubercle. The abundant expression of the rat A2 receptor mRNA in basal ganglia may underlie the known interaction between dopaminergic and adenosinergic systems on behavior.

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617.5 CONTRIBUTION OF THE SECOND ADENOSINE GROUP TO THE TOTAL BINDING OF BIS(6-ADENOSYL)-DECANE TO THE ADENOSINE A1 RECEPTOR. L. A. Havyak and P. M. Collins, Jr., Dept. of Pediatrics, School of Medicine, University of Maryland at Baltimore, Baltimore, MD 21201

Bis(6-adenosyl)-decane is a "bivalent" analog of adenosine, in which the two adenosine groups are separated by a spacer of methylene groups. We have previously reported a series of these compounds whose affinities for the A1 adenosine receptor range from approximately 3 to 300 nanomolar. In order to assess the contribution that the presence of the second adenosine group makes to this binding, we have compared one of the best bivalent compounds, bis(6-adenosyl)-decane, with its monovalent counterpart, 6-decyladenosine. Since 6-decyladenosine contains only one of the two adenosine groups, any differences in binding affinity should be attributable to the second adenosine group.


The vasoactive agent CV 1808 ([2-phenylaminoadenosine] has been previously shown to be a selective antagonist for the A2 adenosine receptor (Javins et al., J. Pharmacol. Exp. Ther. 251, 888-889, 1989). CV 1808 was subsequently radiolabeled and was evaluated in membranes prepared from Sprague-Dawley rat striatum, cortex and hippocampus. Specific binding accounted for 65-70% of the binding in the three brain areas with IC50 values of 16-24 nM, and 25-37% of the binding in IC50 values of 590-1150 nM. The adenosine A1 selective compounds, CPA and CADO, showed 0% of total specific binding with IC50 values of 270-1750 nM and 960-2560 nM, respectively. Interestingly, the adenosine A2 selective agonist CGS 21680 and the non-selective agonist NECA and MECF inhibited < 20% of total specific binding at a concentration of 10 μM. In contrast, CGS 15943, an adenosine antagonist, inhibited 56-68% of total binding, with an IC50 value of 128-310 nM. In addition, several novel adenosine analogs, such as CGS 22988, inhibited 100% of specific binding with IC50 values of 370-430 nM in the three brain areas. The adenosine uptake inhibitor, dipryridamole, and the guanine nucleotide analog, GTPγS, were unable to inhibit the binding of [3H]CV 1808. These results suggest that [3H]CV 1808 labels two binding sites in rat brain, neither of which appear to be the adenosine A2 receptor. One site appears to be an adenosine A1-like component, whereas the second site appears to be a novel adenosine binding site. Further studies to identify a functional correlate for this novel binding site are presently in progress.

617.7 TRANSPORT KINETICS AND METABOLISM OF ACCUMULATED [3H]-ADENOSINE AND [3H]-L-ADENOSINE IN RAT CEREBRAL CORtical SYNAPtoNEURoneS. J. G. Gu, A. N. Sawka* and J. O. Cogger, Dept. of Pharmacogenetics, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

[3H]-Adenosine was previously found to be more metabolically stable than [3H]-adenosine and to be a substrate for CNS adenosine transporters [Gu et al., J. Neurochem. 58 (1991) 348-352]. Here we examined the degree to which accumulated [3H]- and [3H]-adenosine were metabolized and the effects that this metabolism had on transport characteristics. For [3H]-adenosine, there was an inverse relationship between transport reaction times and Km values; for 5, 15 and 600 sec there were 24, 32, and 76% conversions to nucleotides; the Km values (μM) were 9.4±2.2, 8.4±2.3, and 4.5±0.6, respectively. Ten minute incubations of tissue with 10 μM EHNA, an adenosine deaminase inhibitor, and 5'-iodotubercidin, an adenosine kinase inhibitor, resulted in significantly higher Km values of 36±8 μM for 5 sec and 44±13 for 15 sec incubations; metabolism of accumulated [3H]-adenosine to nucleotides decreased to 6.4% at 5 sec and 0.9% at 15 sec. In the presence of these inhibitors, 32% of accumulated [3H]-adenosine was metabolized to nucleotides for 600 sec incubations and the value of 18.8±4.7 did not significantly differ from control value of 4.5±0.6. For [3H]-adenosine, Km value for 20 sec incubations were 38.7±6.8. Metabolism of accumulated [3H]-adenosine to nucleotides was 2.5% ±3.4 and 30.9% for 5, 15 and 600 sec incubations, respectively. Metabolism of accumulated adenosine affects measured kinetic parameters for adenosine transport and the Km value for adenosine transport in rat cerebral cortical synaptoneurosomes is approximately 40 μM.

617.8 THE ADENOSINE REUPTAKE ANTAGONIST SOLUFziLANE REDUCES AND DELAYS POPULATION ANOXIC DEPOLARIZATION IN RAT HIPPOCAMPAL NEURONS IN VITRO. C. G. Boussaid and V. Y. Gribov, Neuropharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

Endogenous adenosine, released by hippocampal neurons during hypoxia, mediates hypoxic protection of synaptic transmission, a response which may be neuroprotective (Ushkoff et al. Brain Res. 512, 293, 1990). Application of metabolically stable adenosine analogs prior to an ischemic event results in a decrease in ischemic neuronal death (Evans et al., Neuron Lett. 82,287, 1997), while application of adenosine receptor agonists increases the relative hippocampal injury (Rudolph et al., J. Cereb. Blood Flow Metab. 7, 74, 1987). In the present experiments we have begun to examine the ability of an adenosine reuptake antagonist, solufzilane, to potentiate the potentially protective effects of endogenous adenosine release; this compound has been previously shown to increase the inhibitory effects of exogenously applied adenosine in hippocampal slices (Ashton et al., Eur. J. Pharmacol. 142,403, 1987). Population axonal depolarizations, recorded from stratum radiatum in area CA1 of rat hippocampal slices in response to exposure to N2 gas, were reduced in amplitude in slices incubated in 10 μM solufzilane (to 39.8% of control); this effect was less pronounced at 20 and 50 μM solufzilane, although the higher concentrations of this reuptake antagonist produced a concentration-dependent increase in synaptic inhibition in similar fashion. Lower concentrations of solufzilane (to 274.7% of control). These data suggest that increasing extracellular adenosine levels by blocking reuptake of the nucleoside can ameliorate a potentially cytotoxic neuronal response to hypoxia.


20 micron-sections from autopic samples of the hippocampus and parahippocampal gyrus were cut and thaw-mounted on gelatine-coated slides. After preincubation (30 minutes, 20°C) in 150 nM Tris-HCl buffer pH 7.4 with 1.5 IU/ml ABA the sections were incubated (2 hours, 20°C) with [3H]DPCPX (10-2 to 20 nM) followed by 2 rinses with buffer (4°C, 120 sec). Non-specific binding in the presence of 20 nM R-PIA was 15%. Kinetic parameters as determined by relating the incubated sections off the slide and liquid scintillation spectrophotometry were Kt=1.6 μM and Bmax=165.9 fmol/section.

Saturation analyses using quantitative autoradiography and determination of cell density in adjacent Galloeynin-stained sections provided receptor fine cell density in subregions and individual layers of hippocampus.

617.10 RADIOLIGAND BINDING STUDIES OF CGS 21680 TO ADENOSINE RECEPTORS IN SPINAL CORD AND STRIATAL MEMBRANES. V. Pisac, H. K. Fouad, J. I. Choca, Dept. of Pharmacology, Univ. of Illinois College of Medicine at Chicago, Chicago, IL 60612.

To better characterize adenosine A1 receptors in the spinal cord we performed parallel studies in membranes of spinal cord and striatum. In both preparations CGS 21680 displaced [3H]NECA from two sites with similar Ki values (Ki=374 and 22.0±2.4 μM; Ki=6.4 and 4.0±0.4 μM for cord and striatum, respectively). In the spinal cord 30% of these [3H]NECA sites have characteristics of adenosine A1 receptor. Although displacement of [3H]NECA has a Ki of 374 μM with R-PIA, CPA, CADO, DMA, DMPX, confirmed that 12 to 30% of the [3H]NECA binding sites show characteristics of an adenosine A1 receptor. However we have been unable to detect direct binding of [3H]CGS 21680 to spinal cord membranes using concentrations in the range of Kt (4 to 80 nM). At higher concentrations (30 to 400 nM) [3H]CGS 21680 binds to a site which has a Kt of 125 μM. Various displacement studies (CGS 21680, DMA, CPA, CADO) suggest that 90 to 99.9% of this binding is to a site which has characteristics of adenosine A1 receptors. The remaining 0.1 to 9.9% of the displacable binding appears to be an A2-like receptor.

These conflicting results suggest that A1 receptors in the spinal cord differ from those in striatum. Perhaps the binding kinetics of [3H]CGS 21680 are much faster in the spinal cord than in the striatum. Faster kinetics could explain our inability to observe direct binding. (Supported by USPHS Grant DA03980)
617.11 EFFECT OF NEONATAL CAFFEINE ON ADENOSINE A1 RECEPTOR ONTOGENY USING AUTORADIOGRAPHY. Barbara A. Etzel and Ronnie Guille, Department of Psychology, University of Rochester, NY 14627.

Caffeine (C), an adenosine antagonist, is commonly administered as treatment for apnea of prematurity in infant humans. To investigate the long term effects of this treatment, an immunogold assay of 14-19 day old rats was performed. The brain was shown to have neonatal C exposure significantly increased specific adenosine A1 receptor (AIR) binding in cerebellum (CBL) and hippocampus (H), two areas in which AIR binding is not normally seen. Specific binding is decreased in the older stages of development. Sections were then incubated with 1 nM 3H-cyclohexyladenosine (3H-CHA). Nonspecific binding was determined in adjacent sections with L-phenylisopropyladenosine. The sections were then processed for LKB Ultrascan for 4 weeks. Quantiﬁcation was performed using Analytical Imaging Concepts software.

617.12 PENETRATION OF ADENOSINE ANTAGONISTS INTO MOUSE BRAIN AS DETERMINED BY EX VIVO BINDING. K.A. Jacobson,* O. Nikiforov,† and J. Baumgold,* †NIDDK, NIH, Bethesda, MD, †Dept. of Radiology, George Washington Univ., Washington, DC.

The penetration of peripherally-administered adenosine antagonists into mouse brain was determined using ex vivo binding and autoradiography in naive mice after a single intraperitoneal (IP) injection. CPT (8-cyclopentyltheophylline), CPA (8-cyclopentyl-1,3-dipropylxanthine), 8-PT (8-p-sulfophenyltheophylline), and N-acetylbenzoylcycteine (NAC) were administered at 10 min post-injection (IP), CPT and CPA (25 mg/kg and 0.25 mg/kg, respectively) both had penetrated into brain substantially; 49 and 17 % of theoretical levels (assuming free penetration throughout the body), respectively. Brain levels of CPA decreased rapidly, declining to undetectable levels by 30 min post-injection, whereas levels of CPA increased to 51% penetration at 20 min, then declined slowly. As expected, no detectable brain levels of 8-PT were found following intraperitoneal injection of 50 mg/kg. XAC (20 mg/kg) penetrated into brain poorly: 1.6% of theoretical at 10 min and 3.2% 20 min post-injection. As a control for vascular content of brain tissue, 14C-bovine serum albumin was administered iv., and the level of radioactivity found in the brain was 1% of that found per equal volume of plasma (corresponds to 1:1 penetration). The ability of CPT to stimulate locomotor suppression paralleled the brain levels, ie. it was similar to theophylline at short times, and the effect rapidly diminished. These studies demonstrate the usefulness of ex vivo binding in determining CNS penetration of adenosine receptor ligands.


Phorbol esters and adenosine are potent modulators of neuronal activity in the central nervous system. In the CA1 region of hippocampus, activation of protein kinase C (PKC) by phorbol esters results in an increase in neuronal activity, whereas activation of adenosine A1 receptors inhibits neuronal activity. Previous studies indicate that these systems can interact. Phorbol esters attenuate the inhibitory action of adenosine on population spike responses in the hippocampus (Rind & et al., J. Physiol. 498, 1997). The present study examined the influence of phorbol esters on two physiological actions of adenosine mediated by A1-type receptors.

In vitro hippocampal slices from adult gerbils were utilized to record: 1) orthodromically-evoked, synaptic responses. Blocking of an inhibitory component of CA1 fEPSPs by low calcium was enhanced by PDAc and PDBu while PDD was ineffective. However, by 30d, binding in C-ex rats equalled or exceeded that in controls. In CBL, more labelling was apparent in the molecular layer (M) than in the granular layer (G); especially in the older rats. The layers could not be distinguished in the 14d C-ex group.

617.14 SELECTIVE CHANGES OF ADENOSINE RECEPTORS IN RAT BRAIN SLICES FOLLOWING IN VITRO EXPOSURE TO RECEPTOR AGONISTS M.P. Abbracchio, A.M. Paoletti*, B. Paternieri* and F. Cattabeni. Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy.

Adenosine is known to physiologically inhibit excitatory neurotransmission in the CNS by activation of specific receptor subtypes. This is believed to be at the bases of neuroprotection by adenosine analogues against ischemia- and excitatory amino-acid-induced cerebral damages. However, there are indications that adenosine neuroprotection might be reduced when brain adenosine concentrations are markedly and pathologically increased (e.g., prolonged ischemia and aging). To verify whether such possible losses of adenosine neuroprotective activity might be due to agonist-induced desensitization of cerebral receptors in consequence to the prolonged exposure to adenosine, we have studied the regulation of adenosine receptors in rat brain slices exposed for different time-periods to selective adenosine receptor agonists, such as cyclo-pentyl-adenosine (CPA) and N-ethylcarboxamido-adenosine (NECA). After exposure of slices to 0.1-10 uM CPA for up to 1 h, the ability of adenosine analogues to inhibit membrane adenylate cyclase activity and cAMP production was decreased and time-dependently reduced, suggesting a loss of A1 receptor function. Desensitization of A1 receptors with NECA was slower in onset and less pronounced, according to the lower A1 receptor selectivity of this adenosine analogue with respect to CPA. Moreover, A2 receptors seemed to be less affected by both agonists in the experimental conditions used, resulting in an imbalance between A1 and A2 receptor function, which might be of functional relevance to ischemic conditions.


The release of endogenous adenosine (ADO) and inosine (INO) from rat hippocampal slices was investigated by HPLC. Recovery of stable evoked population spikes, recorded from the CA1 region, occurred within 150 min from cut and ADO and INO release were stable and reached 14.2±1.5 and 16.0±2.1 nM respectively. Ischemia was then produced by superfusing the slices with glucose free Krebs saturated with 95% N2/5% CO2 for 5 min. The ischemia-evoked release of ADO and INO was respectively 13±2.37 and 10±2.6 nM respectively. Electrophysiological and biochemical responses disappeared within 3 min of ischemia; complete recovery occurred in 4 min after returning to normal oxygenated Krebs solution. Release of ADO and INO evoked by 5 min ischemia was reduced by 62% and 39% respectively in the presence of TTX (0.5 uM) and by 26% and 20% in the presence of the NMDA receptor antagonist D-AP5 (100 uM). The results indicate that ischemia-evoked release of ADO and INO is greatly dependent on influx of Na+ through voltage dependent Na+ channels activity and partly mediated by activation of NMDA receptors. (C.N.R. and University Grants).

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617.17

Adenosine is a neuromodulator which may play a physiological role in the enteric nervous system to modify gastrointestinal motility. We have investigated the relative importance of the inhibition, by adenosine analogs, of the evoked release of Substance P (SP) and Neurokinin A (NKA) from nerves in the myenteric plexus of guinea pig ileum. The inhibition of field-stimulated contraction of guinea pig longitudinal muscle/myenteric preparations. Identification of the subtype of adenosine receptor mediating this response was sought. Inhibition of contraction of field stimulated U0126 was observed for all adenosine analogs: the A1-selective agonist adenosine (CPA) being more potent than the A1-selective agonist which included CGS-21680. Antagonism of the responses with the A1-selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and subsequent Schild analysis revealed, for CPA, a slope of 0.971 and a pA2 of 1.15±0.01. The same analysis for the A1-selective agonist yielded slopes that were not significantly different from zero. Direct measurement of the inhibition of release of SP and NKA from perfused enteric ganglia yielded similar results. These data provide evidence that adenosine A1 receptors functionally coupled to the release of excitoratory mediators. The consistent micromolar CP84, obtained for the A1 agonists suggest either a high threshold activity at A1 receptors or action at putative distinct A1 receptors. The lack of antagonism of A1 agonists by the A2 antagonist DOPA suggests that the former possibility may not involve simple competitive interaction. Supported by NHC of Canada.

617.19
N^6-CYCLOPENTYLADENOSINE, A STABLE ADENOSINE ANALOG, EXERTS CONCENTRATION DEPENDENT EFFECTS ON EVOKED DENTATE GRANULE CELL ACTIVITY. T.H. Svensson and L.M. MacDowall, Dept. of Neurology, Graduate Hospital and Institute of Neuroscience, Univ. of Pennsylvania School of Med., Philadelphia, PA 19104.

Adenosine is involved in intercellular signalling in the hippocampus, although in what capacity is not entirely clear. Inhibitory actions of adenosine have been identified in regions CA3 and CA1, but relatively little is known about its role in the dentate gyrus. Since the dentate is an important "gateway" to the hippocampus, we chose to characterize the role of adenosine in this region. Standard microelectrode field recordings were performed on freely-moving rats utilizing a variety of adenosine analogs; the A1-selective agonist N6-cyclopentyladenosine (CPA), a stable, A1-receptor specific adenosine analog, at several concentrations. CPA at concentrations and above 10 nmol (nM) reversibly inhibited the PSPs and AMPA receptor mediated responses. The inhibition was concentration-dependent and was seen in a variety of cells from different preparations. CPA resulted in a decrease in the number of failures and an increase in the number of successes. These data provide evidence that adenosine A1 receptors functionally coupled to the release of excitatory mediators. The consistent micromolar CP84 obtained for the A1 agonists suggest either a high threshold activity at A1 receptors or action at putative distinct A1 receptors. The lack of antagonism of A1 agonists by the A2 antagonist DOPA suggests that the former possibility may not involve simple competitive interaction. Supported by NIDA grant DA 02702 and the Veterans Admin. Medical Research Service.

617.20
UNITARY EPSPS MEASURED BY WHOLE-CELL RECORDING ARE REDUCED BY ADENOSINE IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS IN VITRO. T.V. Dandridge, C.R. Lupitus, and W.R. Proctor, Veterans Administration Medical Research Service and University of Colorado Health Sciences Center, Denver, CO 80262.

Adenosine is an inhibitory neuromodulator of synaptic transmission in the central nervous system. Although indirect evidence indicates that adenosine acts at least in part by reducing transmitter release, direct evidence regarding the actual pre- and postsynaptic actions are lacking. In the present experiments, we have characterized the effects of adenosine on excitatory responses at excitatory Schaffer collateral and commissural synapses onto hippocampal CA1 pyramidal neurons. Unitary EPSPs generated by low intensity stimulation (minimal stimulus close to the threshold at a high frequency) were recorded with an elevated bath pressure which is the most likely the result of quantal release of the excitatory transmitter. The stimulus voltage was set so that in 10 - 30% of the trials there was no apparent response ("failures"), i.e., the amplitude of the response was smaller than the baseline noise (std. dev. of noise estimates was 50 μV). Superfusion of adenosine (20 μM) resulted in a profound reduction in the mean EPSP amplitude and an increase in the number of failures. The ratio of variance/mean EPSP amplitude for these responses was virtually unchanged by adenosine, and was the same for both the 1st EPSP and the 2nd potentiated EPSP elicited with paired pulses (50 μs interval). These data are consistent with the hypothesis that the major effect of adenosine in this preparation is to decrease the amount of transmitter released from excitatory presynaptic nerve terminals. Supported by NIDDK grant DA 02702 and the Veterans Admin. Medical Research Service.

617.22
ADENOSINE MODULATES PURINERGIC MOTOR TRANSMISSION IN RAT URINARY BLADDER. J. Pablo Ruidobro-Toro, C. Gloria Acevedo, E. Contreras and G. Bustos, Neurosurgical Regulation Unit, Dept. Physi ology, Faculty of Biological Sciences, P. Cath olic Univ. Chile and Dept. Pharmacology, Faculty of Biological Sciences, CHILE.

To examine whether adenosine (A) modifies the purinergic motor tone of the bladder, experiments were conducted in isolated bladder strips superfused with buffer containing atropine plus guanethidine; tissues were electrically stimulated. A and related structural analogs blocked the electrically induced twitches (0.1-5 Hz) in a concentration-dependent fashion. The rank order of potency was R-PiA > CHA > N ECA > A > S-PIA. A also reduced the ATP-induced contraction (NECA > CADO > R-PIA > A > CHA). The application of 8 phenyl theophylline (8PT) increased the magnitude of the twitch response without modifying the basal activity; 8PT antagonized the A-induced inhibitory effect. The ATP-adapted contractions. Results support the hypothesis of a physiological regulatory A tone in the rat bladder mediated via A1 and A2 receptors acting at pre and postjunction sites respectively. FONDECYT Grants #699-90 and 767-90.
DIFFERENTIAL DISTRIBUTION OF PURINE DEGRADATIVE PATHWAYS BETWEEN GLIAL (G) AND NEURONAL (N) CELLS IN CULTURE. G. CERALLO, R. W. J. CHATTERJEE AND J. P. WATTS. Departments of Pharmacology and Physiology, University of Virginia, Charlottesville, VA. 22908.

Degradation of adenosine (ADO) in nervous tissues appears to occur in G but not in N. To demonstrate that the case we performed studies in cultured embryonic chick G and N cells from ciliary ganglia. Cells were incubated in Krebs-Henseleit solution containing saturating concentrations (10^-4 M) of the exogenous substrate for each degradation pathway: xanthine oxidase (XO) for adenosine deaminase (ADA), inosine (INO) for nucleoside phosphorylase (NPP), and hypoxanthine (HYP) for xanthine oxidase (XO). Accumulation in the bathing media (nmol/10^7 cells) of the products of each of these enzymatic reactions were evaluated by HPLC. Accumulation of UMP+UA measured AOD+HPP+UA measured NF and UA measured X0.

As far as X0, the accumulation of X0 rates with time and is greater in G than in N (Fig). These results show that XO is preferentially found in G cells and is almost absent in N. The same patterns was found for ADA and HPP.

These results demonstrate that there is a differential distribution of purine degradative pathways in G. In addition our results also demonstrate, for the first time, the presence of X0 in G. Supported by the National Inst. of Cardiol., I.P.N. School of Med., Mexico and a grant giving grant Johnson & Johnson.


The biosynthesis of catecholamines, such as epinephrine and norepinephrine, as well as peptide hormones, such as vasopressin and oxytocin, within secretory vesicles requires reducing equivalents from an aerobic. To maintain the levels of ascorbic acid within these vesicles, reducing equivalents are shuttled to the ascorbic via a trans-membrane protein, cytochrome b561. We have recently analyzed the kinetics of the individual reactions between cytochrome and the cytochrome b561 on the internal and external surfaces of the vesicle membrane and hypothesized that the reaction between cytochrome b561 and ascorbate involves the concerted transfer of both a proton and an electron (J.R. Biol. Chem. 266, 6879 (1991)). Since histidine has a pK near neutrality and might participate in proton transfer, we tested compounds which react with histidine for effects on the electron transfer properties of the cytochrome. We now report that diethylpyrocarbonate, a chemical modifier of histidine residue, dramatically slows the rate of electron transfer from ascorbate to oxidized cytochrome b561. The inhibition is greater at higher pH, and the electron transfer is completely blocked at pH 8.5. This argues that at least one of the seven histidines in cytochrome b561 participates in the electron transfer process and supports the hypothesis of concerted electron/proton transfer. Supported by NIH grant GM 33849 and the American Heart Association.

RELEASE OF AN ENDOSKEW LIGAND FOR THE CANNABINOID RECEPTOR FROM RAT BRAIN SLICES. D. M. Evans, M. R. Johnson and A. C. Howlett. Dept. Pharmacological and Physiological Science, Saint Louis University, 1402 S. Grand Blvd., St. Louis, MO 63104.

Although marijuana and cannabinoid drugs have been used for medicinal purposes for centuries, only recently has the cellular mechanism of action - namely inhibition of adenosine cyclic via a G-coupled receptor - been elucidated. Binding of the synthetic ligand [3H]CP55940 allowed the characterization of the cannabinoid receptor. This receptor has been shown to exist in brains of many animal species and has recently been cloned. However, to date, an endogenous ligand for this receptor has not been identified. The localization of receptors on neurons may suggest a role in modulation of ripons and hence would indicate a possible neurotransmitter-like molecule as the endogenous ligand. In order to investigate whether an endogenous ligand is present in rat brain and can behave in a similar fashion to "classical" neurotransmitters we examined the ability of the calcium ionophore A23187 to release a compound which could subsequently interact with the cannabinoid receptor on rat brain membranes. We report that release of inhibitory activity was dependent on both concentration of A23187 and time of exposure to the ionophore. Because many classical transmitters as well as eicosanoid metabolites are released in response to such treatment, a large number of these compounds were analyzed for their abilities to inhibit [3H]CP55940 binding. Results from these studies are reported.

Supported by NIDA grants DA03609, DA03612 and Glaxo, Inc.
618.5 METHIONINE INCORPORATION AND TAURINE CONTENT DURING DEVELOPMENT. P.-L. Liu* and R.J. Huxtable. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona 85724.

Huxtable et al. (Neurochem. Res., 15:233, 1990) found an inverse correlation between the increase of the phosphorylated system of the thromboxane A2 receptor and the decrease in taurine content in rat brain P.B fraction during development. However, Schaffler et al. (FASEB J., 5:591, 1991) found that, in the rat, taurine inhibits the methyltransferase, the enzyme converting phosphorylated system to the thromboxane A2 receptor. These observations led us to study the incorporation of [H]-methionine in rat brain P.B fraction during development to determine if a relationship exist between taurine concentration and the incorporation of [H]-methionine into phospholipid from rat brain. Seven, 14, 21 and 28 days-old rat were injected i.p. with 300 μCi/kg [H]-methionine. At 9 h, the amount of radioactivity was determined in the P.B fraction (protein, supernatant and phospholipid). Compared to younger rats, 28 days-old rats have significantly increased incorporation of [H]-methionine into phospholipid, with no change in incorporation into protein. In general, incorporation is inversely correlated with taurine concentration. These results suggest that taurine alters the phospholipid membrane composition which in turn may affect taurine modulation of Ca+ binding to membranes.


A variety of neuroepithelial tumors are involved in the central control of gastrointestinal (GI) functions. Amongst these peptides are cholecystokinin (CCK) and gastrin-releasing peptide (GRP). A large number of studies indicate that these two peptides function as neurotransmitters in the brain. A model of the CCK and GRP central effects rely directly to GI tract functions and control, such as effects on gastric acid secretion, satiety and feeding behavior. The role which these peptides play linked to the mechanisms which regulate acid secretion, satiety and feeding behavior.

Using these improved assay conditions the concentration of CCK/GRP was determined in the SK-N-MCIXC cells. CCK/GRP were detected by region specific RIA experiments performed. CCK/GRP were found in the SK-N-MCIXC cell line and a large number of studies indicate that these two peptides function as neurotransmitters in the brain. A model of the CCK and GRP central effects rely directly to GI tract functions and control, such as effects on gastric acid secretion, satiety and feeding behavior. The role which these peptides play linked to the mechanisms which regulate acid secretion, satiety and feeding behavior.

619.1 ANALYSIS OF SINGLE ADRENAL MEDULLARY CELLS BY REVERSED PHASE MICROCOLUMN LIQUID CHROMATOGRAPHY. B.R. Cooper, J.A. Jankowski, D.J. Lechmanski, R.M. Nigman, J.R. Jagodska. Dept. of Chemistry, Univ. of North Carolina, Chapel Hill, NC 27599-3290.

The increasing need to analyze small sample amounts has prompted development of miniaturized liquid chromatography designs. Microcolumn liquid chromatography columns not only are capable of handling low ng injection volumes, but they offer higher separation efficiencies than conventional sized columns. A microcolumn liquid chromatography column with electrochemical detection was used to quantitatively determine the total amount of epinephrine (Epi) and norepinephrine (NE) in single cultured adrenomedullary cells. The percent release of Epi and NE by a single cell due to a chemical stimulation has also been determined. A single cell was exposed to acetylcholine or carbachol, and an aliquot of the celluar bathing solution was removed and analyzed. Then the stimulated cell was lysed, and the remaining solution was analyzed.

619.2 INTRICACIES OF HANDLING 4-HYDROXY-AND DETERMINING RESERPINE BINDING PARAMETERS. J.D. Seegmiller and B. Bjella. Dep. of Pharmacology, Univ. of Arkansas Medical School, Little Rock, Arkansas 72205.

Reserpine has been used extensively for both therapeutic and research purposes and as a research tool for defining catecholamine stores and to identify the catecholamine transporter in the membranes of storage vesicles found in adrenergic nerve endings and in the chromaffin cells of the adrenal gland. One of the difficulties of working with reserpine is that it sticks extensively to both glass and plastic surfaces. In the presence of less than 5% of the total reserpine in a binding assay bound to glass test tubes, the reserpine was found to be 20% bound to the test tubes during an hour. The results show that the nature of the reserpine binding to the test tubes is less than 5% bound to the test tubes. The reserpine binding to the test tubes is less than 5% bound to the test tubes. The reserpine binding is also less than 5% bound to the test tubes.

619.3 ION DEPENDENCIES OF SEROTONIN TRANSPORT IN A TRANSFECTED CELL MODEL. Albert S. Chang* and Dominic Man-Kit Lam. Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

A clonal, transfected cell line, L-SI, which stably manifests high-affinity serotonin transport properties, was produced by transfection of human genomic DNA into mouse L-M fibroblasts. Using this cell line, we analyzed imipramine-sensitive, 5-HT binding to the transport system. This binding activity was not attributable to the presence of 5-HT receptor subtypes in L-SI cells, and was Na+ independent but Cl- dependent. In contrast, 5-[3H]5-HT transport was both Na+ and Cl- dependent for these cells. The apparent Km, but not the apparent Vmax, was varied with extracellular concentrations of either ion. The coupling ratio for 5-HT, Na+ and Cl- was deduced to be 1:1:1 per transport cycle. By postulating that both Na+ and Cl- ions facilitate 5-HT transport by directly interacting with, or binding to, the transport system along with 5-HT, we used kinetic modelling to account for the involvement of each ion in this transport process. Both binding and transport results supported an ordered sequence of Cl- and 5-HT binding to the transporter (Cl- first, and may facilitate 5-HT binding); Na+ binding is not ordered relative to Cl- binding, but is necessary for membrane translocation of 5-HT.

619.4 KINETIC AND ALLOSTERIC MODEL FOR THE ACETYLCHOLINE TRANSPORTER-VESAMICOL RECEPTOR. E.D. Clarkson, B.A. Bahr, G.A. Rogers and S.M. Parton. Neuroscience Research Institute, Univ. of California, Santa Barbara, California 93106.

The ligand binding relationship between the acetylcholine transport carrier (AcChT) and the vesamicol receptor (VR) in synaptosomal vesicles purified from the Torpedo electric organ was studied using analogs of AcCh and vesamicol. AcCh inhibits binding of [3H]vesamicol with an affinity much lower (200-fold ratio) than its affinity for active transport (Km = 0.3 μM). Three analogs of AcCh that are up to 50% larger in volume than AcCh and also exhibit large ratios for the macroscopic competitive dissociation constants measured by inhibition of vesamicol binding and active transport of AcCh, analog 15 (N,N-dimethyl-3-pyrrolidinyl benzamide iodide), that exhibits a ratio of 25, was synthesized in tritiated form and shown to be actively transported with the same Kmax as AcCh itself. The transport mechanism of trans-6-methoxyvesamicol was shown to be a noncompetitive inhibitor of AcCh and analog 15 active transport. The data demonstrate that vesamicol binds to an allosteric site in the AcChT. A specific model is proposed, and values for some of the microscopic constants in the model are estimated. The model posits very loose binding of AcCh to the AcChT at 40 μM which is followed by rapid transport. Little selectivity for the structure of the transported ion is exhibited.
619.5 EFFECTS OF LOCAL ANESTHETICS/COCAINE ON CHOLINE UPTAKE. Merouane Bencehrif and Ronald J. Lukas. Division of Neurology, Bascom Palmer Eye Institute, University of Miami, Miami, FL 33101.

Cells of the TE67/1B/BD human clone and the PC12 rat pheochromocytoma exhibit a number of neuron-like features including the association of high affinity choline uptake (HACU) with lipid rafts. IC50 values for blockade of HACU in the presence of 10μM [3H]-choline at 20°C are 1μM for native choline and 2μM for hemicholinium-3. By contrast, IC50 values are 100 and 1000μM, for nicotine and carbamylcholine, respectively. HACU is also sensitive to blockade by local anesthetics dimethisquin (5μM), teicoplanin (50μM) and procaine (500μM) at concentrations comparable to those that inhibit ion flux through nicotinic receptors expressed by TE67/1B and PC12 cells (IC50 values of about 3, 3, and 40 μM, respectively). These observations suggest that local anesthetics at therapeutic doses may not only affect ligand-gated and voltage-gated ion channel function, but may also affect HACU, which is the principal regulatory factor in acetylcholine synthesis. Furthermore, the local anesthetic cocaine inhibits HACU by TE67/1B and PC12 cells with IC50 values of about 500μM, suggesting that anomalous parasympathetic effects of cocaine and the observation that behaviorally-relevant and lethal doses of cocaine exceed those known to inhibit high affinity catecholamine uptake may both be explained by heretofore unrecognized effects of cocaine on acetylcholine metabolism and HACU.

619.7 INHIBITION OF THE REUPTAKE OF NOREPINEPHRINE AND SEROTONIN. CONFORMATIONALLY CONstrained ANALOGUES OF AMPHETAMINE, A SELECTIVE MONOAMINE REUPTAKE INHIBITORS. Gary L. Grunewald, David W. Lewis*, and Sheila M. Zipfel*. Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045.

While some antidepressant drugs show selectivity in the inhibition of reuptake of either serotonin (5-HT) or norepinephrine (NE), no clear relationship between the structure of the antidepressant drug and the neurotransmitter selectivity has been shown. Previous molecular mechanics calculations in our laboratory (J. Med. Chem. 1988, 31, 913-919) have suggested a correlation between the antidepressant activity and the conformation of key portions of flexible amine reuptake inhibitors. To probe this point, we have synthesized several conformationally constrained monoamine reuptake inhibitors which have been designed to mimic calculated low energy conformational models of tricyclic antidepressants. The new conformationally constrained analogues have been evaluated for selectivity in the inhibition of the uptake of NE and 5-HT into rat cerebral cortex. The selectivity ratio (ratio of IC50 values for inhibition of the uptake of NE compared to 5-HT) was determined for each compound and compared to the conformationally flexible parent drug. We will discuss our preliminary results, which show that the synthesis of conformationally constrained analogues may be of value to probe the effects of conformation on the selectivity of neurotransmitter reuptake by antidepressant drugs. Supported by NIH training grant GM 9775 and by NSF REU grant CHE 910476.

619.9 DORSOVENTRAL STRIATAL DIFFERENCES IN STIMULATED DOPAMINE (DA) OVERFLOW: RELATIONSHIP TO NONISENSIVE EFFECTS. D. Bushik and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Relative to the caudate-putamen (CPu), the nucleus accumbens septal (NAS) has a paucity of high-affinity DA uptake sites, as evidenced by the lower [3H]DA uptake and a lower Bmax for [3H]DA binding to the associated recognition sites. This lower density of receptors is suggestive of receptor deactivation, and may be relevant to the production of acetylcholine by cholinergic neurons. We have observed that under the same conditions, the uptake of [3H]DA by NAS is 53% greater than that from CPu. This result is apparently due to regional differences in uptake site density, since stimulated overflow was at least 10 times greater in the presence of 10 μM nomifensine. Nomifensine potentiated overflow in both NAS and CPu; however, potentiation of CPu overflow was 1.9 times that of NAS. The results suggest that a given amount of terminal depolarization will cause greater overflow in ventral striatum than in dorsal due to differential transmitter reuptake, while the dorsal striatum may be preferentially affected by DA-reuptake blockers.


Sodium-dependent high affinity choline uptake (SDHACU) is believed to be the rate-limiting mechanism in the production of acetylcholine by cholinergic neurons. We have discovered that a neurotrophic factor, a fraction of an endogenous high affinity choline uptake which is pharmacologically and kinetically similar to the SDHACU of cholinergic sympathetic preganglionic neurons (10μM) and procaine (500μM) at concentrations comparable to those that inhibit ion flux through nicotinic receptors expressed by TE67/1B and PC12 cells (IC50 values of about 3, 3, and 40μM, respectively). These observations suggest that local anesthetics at therapeutic doses may not only affect ligand-gated and voltage-gated ion channel function, but may also affect HACU, which is the principal regulatory factor in acetylcholine synthesis. Furthermore, the local anesthetic cocaine inhibits HACU by TE67/1B and PC12 cells with IC50 values of about 500μM, suggesting that anomalous parasympathetic effects of cocaine and the observation that behaviorally-relevant and lethal doses of cocaine exceed those known to inhibit high affinity catecholamine uptake may both be explained by heretofore unrecognized effects of cocaine on acetylcholine metabolism and HACU.


Dopamine (DA) may diffuse from DA nerve terminals to reach, via extracellular fluid pathways, DA receptors on prolactin producing cells, and reach a neural synapse to modify an event in the cell. We have therefore tested the hypothesis that DA may act as a neuromodulator at any of these remote sites, and that DA may diffuse through neural synapses to replace a neurotransmitter that has been lost. To test this hypothesis, we labeled the DA transport receptors with [3H]GBR 12935 and studied the binding inhibition by inhibitors of the DA transporter receptor. In rat brain, [3H]GBR 12935 preparations were incubated with 10 μM [3H]GBR 12935 in the presence of increasing concentrations of DA analogues. A DA analogue with a shallow inhibition curve (IC50 of 1×10−6 M) was selected. The decrease in [3H]GBR 12935 binding by a DA analogue was found to be most relevant to the decrease in [3H]GBR 12935 binding by GBR 12909.

619.11 FRIDAY AM BIOGENIC AMINES: UPTAKE AND RELEASE. 1551

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FRIDAY AM BIOGENIC AMINES: UPTAKE AND RELEASE

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FRIDAY AM BIOGENIC AMINES: UPTAKE AND RELEASE

A lipophilic weak base, amphetamine (AMPH) reduces vesicular pH gradients; this inhibits uptake and increases release of monoamines from storage vesicles (Soler & Rayport, 1987). We examined whether the resulting and cytosolic transmitter reaches the synapse via reverse action of the uptake transporter. Ventral midbrain from P7 rats was dissociated, plated at a density of about 1000 DA neurons, and maintained for 2-3 weeks. For AMPH incubations, we measured about 100 femoles of extracellular DA (release minus reuptake). Extracellular DA increased over 5-fold with 200 μM AMPH. The plasma membrane DA uptake blocker benztpine (BZT; 10 μM) significantly increased extracellular DA (release alone). When normalized for the effect of BENZ on reuptake, BENZ attenuated the AMPH-induced increase in DA.

619.12 REVERSE ACTION OF THE PLASMA MEMBRANE TRANSPORTER MEDIATES WEAK BASE-INDUCED DOPAMINE RELEASE: IMPLICATIONS FOR AMPHETAMINE ACTION. N. E. O. U. C. M., Rootstown, OH 44272, and University Hospitals, Cleveland, Ohio.

619.13 THE AROMATIC AMINO ACID DECARBOXYLASE INHIBITOR, NSD-1015, INCREASES DOPAMINE RELEASE IN VITRO FROM SUPERFUSED CORPUS STRIATAL TISSUE FRAGMENTS OF MALE RATS. D. E. Dluzen and J. L. McAllister. Departments of Anesthesiology and Medicolegal Medicine, N.E.O.U.C.O.M., Rootstown, OH 44272, and University Hospitals, Cleveland, Ohio.

619.14 ONGOTGENIC STUDIES ON BINDING SITES FOR DOPAMINE UPTAKE BLOCKERS IN NIGRO-STRIATAL DOPAMINERGIC NEURONS. M. Vaclhar1, A. G. Wright, Jr., and I. Hanger. Lab. of Chemical Pharmacology, National Heart, Lung, and Blood Institute, Bethesda, MD 20892.

The expression of dopamine uptake sites that are sensitive to dopamine uptake blockers is developmentally linked to neurite outgrowth. Our results show that in embryos and in newborn rats the binding sites for dopamine uptake blockers appear not to be colocalized with the dopamine transporter in plasma membranes. In 19-day-old embryos and in newborn rats specific binding sites for 123I-GBR-12935 can be measured in intact striatal synaptosomes; but are not detectable in washed synaptosomal membrane preparations.

619.15 AGONISTIC AND ANTAGONISTIC INTERACTIONS OF DOPAMINE UPTAKE BLOCKERS IN THE HUMAN FRONTAL CORTEX. Ana Hetit, Ph.D., Manuel E. Casanova, M.D., Joel E. Kleinman, M.D., Ph.D., and Richard J. Wyatt, M.D. NIMH, National Institute of Mental Health, Bethesda, Maryland.

619.17 123I-σ-PIA ([σ-ISOAMPHETAMINE] MAY LABEL MONOAMINES OXIDASE (MAO) IN CRUDE RAT FRONTAL CORTEX HOMOGENATE: Xueqian Huang*, Michael P. Johnson, David D. Nichols and Chester A. Mathis. Dept. of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Purdue Univ., W. Lafayette, IN 47907. Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

In attempts to develop a novel label for the serotonin uptake carrier, 125I-p-iodoamphetamine ([125I]-P) was synthesized. A specific binding site for [125I]-P was subsequently identified. Scatchard analysis yielded a Kd of 421 ± 16.8 nm, a Hill coefficient of 0.991 ± 0.003, and 6.3 ± 0.1 pmol/mg protein for [125I]-P binding. [125I]-P binding was significantly different from saline treated animals. However, the Bmax of [125I]-P binding was decreased almost 80%. This suggested that the [125I]-P binding site was not the serotonin uptake carrier and was not located on the 5-HT axon terminal. It seemed possible that membrane-bound monoamine oxidase might therefore be the site labeled by [125I]-P. The p-chlorophenylalanine (PCA), d-amphetamine (d-AMP), and 5,6,7,8-tetrahydroxy-1,2,3,4-tetrahydroisoquinolines (5,6,7,8-MDMA) were 184.6 ± 3.5 nM, 32.4 ± 2.8 ng/ml, and 158.6 ± 1.9 pmol/mg protein.

619.16 AGONISTIC AND ANTAGONISTIC INTERACTIONS OF DOPAMINE UPTAKE BLOCKERS IN THE HUMAN FRONTAL CORTEX. Ana Hetit, Ph.D., Manuel E. Casanova, M.D., Joel E. Kleinman, M.D., Ph.D., and Richard J. Wyatt, M.D. NIMH, National Institute of Mental Health, Bethesda, Maryland.

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In attempts to develop a novel label for the serotonin uptake carrier, 125I-p-iodoamphetamine ([125I]-P) was synthesized. A specific binding site for [125I]-P was subsequently identified. Scatchard analysis yielded a Kd of 421 ± 16.8 nm, a Hill coefficient of 0.991 ± 0.003, and 6.3 ± 0.1 pmol/mg protein for [125I]-P binding. [125I]-P binding was significantly different from saline treated animals. However, the Bmax of [125I]-P binding was decreased almost 80%. This suggested that the [125I]-P binding site was not the serotonin uptake carrier and was not located on the 5-HT axon terminal. It seemed possible that membrane-bound monoamine oxidase might therefore be the site labeled by [125I]-P. The p-chlorophenylalanine (PCA), d-amphetamine (d-AMP), and 5,6,7,8-tetrahydroxy-1,2,3,4-tetrahydroisoquinolines (5,6,7,8-MDMA) were 184.6 ± 3.5 nM, 32.4 ± 2.8 ng/ml, and 158.6 ± 1.9 pmol/mg protein.

619.18 123I-σ-PIA ([σ-ISOAMPHETAMINE] MAY LABEL MONOAMINES OXIDASE (MAO) IN CRUDE RAT FRONTAL CORTEX HOMOGENATE: Xueqian Huang*, Michael P. Johnson, David D. Nichols and Chester A. Mathis. Dept. of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Purdue Univ., W. Lafayette, IN 47907. Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

In attempts to develop a novel label for the serotonin uptake carrier, 125I-p-iodoamphetamine ([125I]-P) was synthesized. A specific binding site for [125I]-P was subsequently identified. Scatchard analysis yielded a Kd of 421 ± 16.8 nm, a Hill coefficient of 0.991 ± 0.003, and 6.3 ± 0.1 pmol/mg protein for [125I]-P binding. [125I]-P binding was significantly different from saline treated animals. However, the Bmax of [125I]-P binding was decreased almost 80%. This suggested that the [125I]-P binding site was not the serotonin uptake carrier and was not located on the 5-HT axon terminal. It seemed possible that membrane-bound monoamine oxidase might therefore be the site labeled by [125I]-P. The p-chlorophenylalanine (PCA), d-amphetamine (d-AMP), and 5,6,7,8-tetrahydroxy-1,2,3,4-tetrahydroisoquinolines (5,6,7,8-MDMA) were 184.6 ± 3.5 nM, 32.4 ± 2.8 ng/ml, and 158.6 ± 1.9 pmol/mg protein.
**619.17**

**BW 1370U87 - A SELECTIVE, COMPETITIVE INHIBITOR OF MONOAMINE OXIDASE-A**

H.L. White and P.W. Scates*. Division of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709

BW 1370U87, a potent, selective inhibitor of rat and human brain monoamine oxidase-A (MAO-A) was developed as a potential antidepressant/anxiolytic/antipanic agent. Kinetic experiments demonstrate a competitive mechanism of action for this compound, with Ki = 0.01 μM using either serotonin or tyramine as substrate. After preincubation of BW 1370U87 with mitochondrial MAO, full activity was restored by dialysis, indicating complete reversibility of the inhibition.

Following oral administration to rats, BW 1370U87 inhibited brain MAO-A in a dose-dependent manner, with an ED50 of 8 mg/kg po and a duration greater than 7 hr, but less than 24 hr. No significant inhibition of MAO-B by BW 1370U87 was observed either in vitro or ex vivo. Ex vivo inhibition was also reversed by extensive dialysis of brain homogenates from pretreated rats. The selectivity, reversibility, and competitive kinetics of the inhibition of BW 1370U87 route to improved safety profile with this novel MAO-A inhibitor.

**619.18**

**EFFECT OF 1370U87, BROFAROMINE, AND MOCLOBEMIDE ON RAT BRAIN BIOGENIC AMINES AND THEIR ACID METABOLITES**


Monoamine oxidase (MAO) inhibitors have been shown to increase brain concentrations of biogenic amines while decreasing concentrations of their acid metabolites. It has been suggested that the magnitude of this effect is an indicator of MAO inhibition. When 1370U87, a novel and reversible selective MAO-A inhibitor, moclobemide and brofaromine were given to rats at doses shown to inhibit brain MAO-A by 80%. The effects on brain biogenic amines and their metabolites were quantified 2 and 4 hours after injection. Moclobemide (10mg/kg) and 1370U87 (20mg/kg) induced larger increases in 5HT, NE, and DA and larger decreases in DOPAC, SHIAA, and HVA than did brofaromine (5mg/kg). The effect of 1370U87 (20mg/kg po) on biogenic amines lasted 6 hours significantly (p<0.05) increasing NE, 5HT, and DA while decreasing DOPAC, HVA, and SHIAA. Similar results were found in a dose response study when 1370U87 was given orally at 10, 20, or 40mg/kg.

**619.19**

**BLOOD PRESSURE EFFECTS OF MONOAMINE OXIDASE INHIBITORS IN RESPONSE TO ORALLY ADMINISTERED TYRAMINE IN THE RAT.**

M. S. Carroll*, O. G. Beek*, and B. R. Cooper*. Division of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709

The reversible monoamine oxidase A inhibitors 1370U87, brofaromine, and moclobemide, and the irreversible nonselective monoamine oxidase inhibitor phenelzine were compared for potentiation of the pressor response to oral tyramine. Conscious rats were pretreated with doses of the monoamine oxidase inhibitors sufficient to produce 80% inhibition of brain monoamine oxidase, and then were challenged with orally-administered tyramine. Blood pressure was monitored prior to and after tyramine, and peak pressor responses were compared. At a dose of 15 mg/kg tyramine, the pressor response of 1370U87 was statistically similar to the vehicle control response 616U76, brofaromine, and moclobemide elicited mild tyramine pressor effects, whereas phenelzine resulted in a marked elevation of blood pressure. Higher doses of tyramine elicited blood pressure elevations from all of the monoamine oxidase inhibitors.

**619.20**

**CNS PHARMACOLOGY OF 1370U87: A NOVEL REVERSIBLE SELECTIVE MAO-A INHIBITOR**


1370U87 is a potent reversible selective inhibitor of rat and human brain MAO-A having a competitive mechanism of action and an oral ED50 (ex vivo) of 8 mg/kg. The duration of action exceeds eight hours. The ED50 dose for inhibition of MAO-A (20mg/kg) elevates NE, DA, and 5HT levels in brain without significant potentiating the blood pressure effects of orally administered tyramine. No inhibition of MAO-B with 1370U87 has been observed. 1370U87 is effective in the 5-HTP potentiated pressor test and Porsolt test. The compound also has effects on abnormal behavior produced by early mother/infant separation in monkeys. Autonomic testing with 1370U87 revealed that there are no adverse cardiovascular symptoms in dogs or rats. No pharmacologically significant effects were observed on various isolated tissues, receptors or uptake systems. 1370U87 is representative of a new class of safer reversible MAO-A inhibitors which are expected to create a new generation of agents to use for depression, anxiety conditions, phobias, obsessive compulsive behaviors and borderline personality disorders.

**619.21**

**AUTORADIOGRAPHIC LOCALIZATION OF NOREPINEPHRINE UPTAKE SITES IN RAT BRAIN USING [125I]IODOTOMOXetine**

J. L. Schadetzky, E. S. Littfau, D. B. Mace*, W. K. Robertson, and D. R. Gehlert. CNS and Cardiovascular Pharmacology, Lilly Research Labs, Indianapolis, IN 46225

The norepinephrine (NE) uptake transporter is a primary mechanism involved in the termination of the effects of NE. These sites were originally studied using radioligands which lacked specificity for this site or had high levels of nonspecific binding. We have developed a method for localization of NE uptake sites using a novel radiolabeled analog of the potent and specific NE uptake inhibitor, [125I]iodotomoxetine ([125I]ITX).

A series of biochemical studies were conducted to determine the appropriate conditions for labeling side mounted sections of rat forebrain. Once optimal conditions were determined, labeled sections were exposed against a sheet of Hyperfilm-MR for 24-48 hours. Quantitation of the binding was accomplished using [125I]iodotomoxetine ([125I]ITX) and [125I]iodonecine ([125I]IN). These sites were then labeled with the labeled sections. Following film development, the images were analyzed by densitometry using a MACD (Canada) image analysis system. [125I]iodotomoxetine (5 μCi/ml) was bound to tissue sections with an apparent Kd value of 52 pM and a Bmax of 26 fmol/mg protein. Specific [125I]iodotomoxetine binding was inhibited by low nanomolar concentrations of desipramine or timolol while higher concentrations of paroxetine (500 nM) or GR 196135 (1 μM) did not inhibit binding.

Distribution of binding sites correlated well with the known location of NE containing cell bodies and terminals. Moderate levels of binding were found in the hippocampus, zona incerta, nucleus of the solitary tract, dorsal raphe nucleus, anterior thalamic nuclei, hypoglossal nucleus and ventral tegmental area. Moderate levels of binding were seen in the paraventricular nucleus of the hypothalamus. Low densities were found in the caudate-putamen, globus pallidus and cortical areas. Little localization within the basal cortex.

These results indicate that [125I]iodotomoxetine exhibits high affinity and specificity for NE uptake sites and provides a useful tool for autoradiographic studies by providing rapid exposure of autoradiograms.

**619.22**

**PHORBOL ESTERS CAUSE PREFERENTIAL SECRETION OF NOREPINEPHRINE FROM BOVINE CHROMAFFIN CELLS.**


Phorbol esters stimulate the secretion of [3H]norepinephrine from intact bovine chromaffin cells and potentiate Ca2+-induced secretion from permeabilized chromaffin cells (Poole et al., PNAS 82, 930-934, 1985). To examine the effects of phorbol esters on the secretion of the individual catecholamines, we have assayed catecholamine secretion by HPLC with electrochemical detection. We find that phorbol dibutyrate (PDBu) causes a much greater release of norepinephrine (NE) than of epinephrine (E):
619.23
REAL-TIME IDENTIFICATION OF EPINEPHRINE AND NORPINEPHRINE COSECRETION FROM INDIVIDUAL BOVINE ADRENAL MEDULLARY CELLS BY J. W. Jorgenson* and R. M. Rightmire, Dept. of Chemistry, University of North Carolina, Chapel Hill, N.C. 27599-3290.

Carbon-fiber microelectrodes placed adjacent to individual bovine adrenal medullary cells have been used to monitor release of catecholamines and to distinguish between epinephrine (E) and nor epinephrine (NE). The identity of released catecholamine was determined using cyclic voltammetry at electrodes coated with a thick (>350 nm) Wafion film. The identification is based on the difference in the rates of the E or NE reaction of the oxidized forms of the catecholamines (1). Three types of cells were identified: those which secreted primarily E; those which secreted primarily NE; and those which secreted mixtures of E and NE. Liquid chromatographic analysis of single cells shows that the relative proportions of E and NE in the individual cells is the same as the proportions observed during secretion of those same cells. These findings show that although individual adrenomedullary cells in culture primarily contain either E or NE, they are capable of storing and secreting both catecholamines simultaneously.


620.1

The existence of two corticosteroid receptor systems in the rat brain is well established, however regulation of their expression by corticosteroids remains controversial. The present study examined the effects of RU 28362, corticosterone (CORT) and aldosterone (ALDO) on type II (\(\Delta^{n}\)desamethasone \(\Delta^{n}\)RU 28362) corticosteroid receptor binding in dispersed hippocampal cell cultures derived from animals sacrificed at E19-20 days of gestation. Four days of exposure to 10 nM RU 28362 resulted in a robust down-regulation of type II receptors (-70%) whereas similar exposure with 10 nM of either CORT or ALDO produced a more moderate (40-45%) decrease in type II receptor binding capacity. Scatchard analysis revealed that diminished type II receptor level was due to a decrease in total number of binding sites (Bmax) with no differences in Kd (~0.8nM). Given that both CORT and ALDO decreased type II binding to the same extent, it is possible that this effect is mediated via type I corticosteroid receptors. Furthermore, the calculated EC50 for ALDO in the hippocampal cell cultures is low (~7.4 nM) and thus strongly suggests the involvement of type I receptors. In order to address this question, we treated cultured hippocampal cells with 10 nM ALDO ± 20 nM of either type I receptor antagonist (RU 26752) or type II receptor antagonist (RU 38466). Neither antagonist was successful in blocking the type II corticosteroid receptor down-regulation by ALDO. Surprisingly, incubation with either RU 26752 or RU 38466 on their own produced a decrease in type II binding. Studies are currently underway to further characterize these agonist-like effects on type II receptors via type I and/or type II receptors.

620.2
DIURNAL RHYTHMS OF GLUCOCORTICOID AND MINERALOCORTICOID mRNA EXPRESSION IN THE HIPPOCAMPAL FORMATION: REGIONAL SPECIFICITY AND STEROID DEPENDENCE. I. P. Herman, B.S. McKewn, H. M. Chao, H. Coirini, and S. J. Watson, Mental Health Research Institute, U. of Michigan, Ann Arbor, MI 48109, and the Rockefeller University, New York, NY 10001.

Brain mineralocorticoid (MR) and glucocorticoid (GR) receptor subtypes are exposed to a wide range of diurnal glucocorticoid (GC) concentrations. The availability of MR and GR proteins is clearly essential to integration of GC signals across the circadian cycle. To assess this availability, we have employed semi-quantitative in situ hybridization histochemical analysis of MR and GR mRNA expression across the circadian cycle (4 h intervals) in intact rats and rats deficient of endogenous GCs (adrenalectomized (ADX) rats). MR mRNA showed a significant, bidirectional diurnal rhythm in all hippocampal subfields. ADX resulted in a small (25%) but significant overall increase in MR mRNA in all subfields. GR mRNA, on the other hand, showed a monotonous diurnal rhythm restricted to CA1 and dentate gyrus (DG). Effects of ADX were subfield dependent: pronounced round-the-clock increases were observed in CA1 and CA3 following ADX, whereas in the DG ADX served to eliminate the circadian trough in GR mRNA expression. No circadian or ADX-induced changes in GR or MR mRNA were observed in thefrontoparietal cortex or in the posterior thalamic nuclei. These data indicate that MR and GR mRNA exhibit hippocampus-specific diurnal rhythms of expression which interact with, but cannot be totally explained by, circulating GCs. We hypothesize that hippocampal steroid receptor gene expression is synchronized by both steroid and neuronal input across the daily light-dark cycle. Supported by MH42251, DA02265 and MH41256.

620.3

Using the Xenopus oocyte system as a bioassay for GnRH receptor (GnRH-R) mRNA level, we have previously demonstrated that regulation of the GnRH-R in oocyte pituitary cultures by gonadal hormones is associated with concomitant changes in receptor mRNA levels (Mol. End. 4:119,1990). The transgenic mouse-derived cell line, αT 3-1 cells, express a GnRH-R similar to those found in rat and mouse pituitary (Mol. Endo.5:347,1991), making this cell line an excellent model system for the study of the regulatory mechanisms of the GnRH-R by GnRH.

Exposure of αT 3-1 cells to GnRH induces alterations of GnRH-R mRNA levels, as assayed in oocytes. The effect of GnRH is bidirectional and both time and concentration dependent. Exposure to physiological concentrations of GnRH for 20 minutes cause a significant increase in GnRH-R mRNA at 24 h, but after a continuous 24 h exposure, the mRNA level is unchanged. In contrast, a 20 minute exposure to 1μM GnRH does not alter GnRH-R mRNA at 24 h, whereas 24 h exposure leads to a marked decrease. Preliminary binding data suggest that up- and down-regulation of the GnRH-R mRNA is accompanied by similar changes in receptor number. In rat pituitary, similar effects on GnRH-R biosynthetic rate have been reported (Endo. 126:2577,90). Thus, both homologous and heterologous regulation of the GnRH-R involve modulation of biosynthetic rate. (NIH Grant K11 DK01854)

620.4
REGULATION OF SUBSTANCE P RECEPTOR SYSTEMS BY NALTREXONE. O. J. Lewi, Division of Pharmacology, Schools of Pharmacy/Medicine, UMCK, Kansas City, MO 64108.

Opiate antagonist, naltrexone (NALT), increases opioid peptides in the brain and up-regulates brain opioid receptors resulting in functional supersensitivity, i.e., an enhanced morphine-induced analgesia. SP causes the release of met-enkephalin. Recent evidence strongly suggests that SP system is regulated by endogenous opioid peptides. Here, NALT was used to explore the functional link between SP and endogenous opioid receptor systems. Male Sprague-Dawley rats under ether anesthesia were implanted subcutaneously with Alzet® miniosmotic pumps, filled with either NALT HCI (100μg/ml) or vehicle (control), for 7 days. Animals were decapitated on day 8, whole brains rapidly removed and homogenized in ice-cold buffer for use in in vivo binding assays in order to explore the regulation of SP and intracellular inositol 1,4,5-trisphosphate (IP3) receptors. Using tritiated SP and IP3 as ligands, the affinities (Kd) for SP and IP3 receptors were unaffected by chronic NALT treatment. However, the densities (Bmax) for both SP and IP3 were significantly increased by 35% and 52%, respectively. NALT appears to up-regulate SP receptor. It also appears that IP3 receptor, a calcium mobilizing receptor, is positively modulated by stimulation of other classes of cell-surface receptors.
620.5

UP-REGULATION OF TYPE BENZODIAZEPINE RECEPTOR DENSITY ON THE NB2-MITOGENICALLY-INDUCED ALTERATION OF PERNIPHERAL CELL CLONE. J.M. Evans-Shields* and H.E. Laird II. College of laboratory (Laird et al. 1984) that Nb2 cells possess a τ receptor and that ligands acting on this site modulate proline-stimulated mitogenesis. Therefore, this receptor has a functional role in this system. The Nb2 node lymphomas are unresponsive to GABA receptor function and binding. This might be due to interactions at peripheral benzodiazepine sites on neurons or to indirect effects of compounds such as neurosteroids.

620.6

IMIPRAMINE REDUCED BINDING NUMBER OF SIGMA SITES IN RAT STRIATUM AND HIPPOCAMPUS. V. Shiyama*, T. Nishikawa*, M. Watanabe and K. Takahashi. Natl. Inst. Neurosci., NCPN, Tokyo and Nati. Defence Med. College, Saitama, Japan. Repeated treatment for 14 days with imipramine (IMI; 10mg/kg, i.p., once a day) induced a decrease in the Bmax, without affecting the KD, of [3H]DPG (1,3-di-o-tolylguanidine) binding to the haloperidol-sensitive sigma sites in the striatum and hippocampus of the rat. This reduction dose not seem to be due to the residual IMI in the brain tissues, since lU of IMI in the assay medium of cortical [3H]DPG binding augmented the Kd value, in the absence of change in the Bmax value, of the binding. Finally, repeated treatment with IMI in combination with p-chlorophenylalanine (a serotonin synthesis inhibitor) no longer altered the striatal and hippocampal [3H]DPG binding. These results indicate that the depletion of brain serotonin may antagonize the ability of subchronic administration of IMI to reduce [3H]DPG binding sites in the striatum and hippocampus, suggesting that serotonergic systems could be involved in the regulation of sigma binding sites in these brain areas.

620.7

COADMINISTRATION OF THE PERIPHERAL BENZODIAZEPINE ANTAGONIST PK11195 ATTENUATES LORAZEPAM-INDUCED TOXICITY AND RECEPTOR DOW NDOWNLREGULATION IN NR108-15 L-GABA L.G. Meier, D.J. Greengard* and R.L. Shadley*. Dept. of Pharmacology, Depts. of Pharmacology and Psychiatry, Tufts University. School of Medicine, Boston, MA 02111. Some evidence indicates that ligands at the peripheral benzodiazepine site can influence effects at the central GABA receptor site. The peripheral antagonist PK11195 (PK) co- administered with the benzodiazepine agonist lorazepam (LZ) to evaluate interactions during chronic administration in a mouse model. PK alone (5mg/kg) did not alter motor activity, benzodiazepine receptor binding or GABA-dependent chloride uptake over 14 days. LZ alone (2mg/kg) produced tolerance at 7 days, with accompanying benzodiazepine receptor downregulation in cortex (CX) and hippocampus (HI). The Bmax yield a Kd value of 1.93 ± 0.003 nM and a Bmax of 2.19 ± 0.001 pM for the stationary cells. Log cells yielded a Kd value of 1.54 ± 0.03 nM. These data suggest that homologous up-regulation entails down-regulation followed by an inhibition of receptor degradation.

620.8

CHOLINERGIC INVOLVEMENT IN RENAL PERIPHERAL BENZODIAZEPINE RECEPTOR RESPONSE TO STRESS. P.Y. Holmes & N.C. Drago. Schrier Research Lab. Dept. of Psychology. Brown University, Providence, R.I. Clinical Pharmacology, Dept. of Pharmacology and Psychiatry, Tufts University. School of Medicine, Boston, MA 02111. Some evidence indicates that ligands at the peripheral benzodiazepine site can influence effects at the central GABA receptor site. The peripheral antagonist PK11195 (PK) co- administered with the benzodiazepine agonist lorazepam (LZ) to evaluate interactions during chronic administration in a mouse model. PK alone (5mg/kg) did not alter motor activity, benzodiazepine receptor binding or GABA-dependent chloride uptake over 14 days. LZ alone (2mg/kg) produced tolerance at 7 days, with accompanying benzodiazepine receptor downregulation in cortex (CX) and hippocampus (HI). The Bmax yield a Kd value of 1.93 ± 0.003 nM and a Bmax of 2.19 ± 0.001 pM for the stationary cells. Log cells yielded a Kd value of 1.54 ± 0.03 nM. Some evidence indicates that ligands at the peripheral benzodiazepine site can influence effects at the central GABA receptor site. The peripheral antagonist PK11195 (PK) co- administered with the benzodiazepine agonist lorazepam (LZ) to evaluate interactions during chronic administration in a mouse model. PK alone (5mg/kg) did not alter motor activity, benzodiazepine receptor binding or GABA-dependent chloride uptake over 14 days. LZ alone (2mg/kg) produced tolerance at 7 days, with accompanying benzodiazepine receptor downregulation in cortex (CX) and hippocampus (HI). The Bmax yield a Kd value of 1.93 ± 0.003 nM and a Bmax of 2.19 ± 0.001 pM for the stationary cells. Log cells yielded a Kd value of 1.54 ± 0.03 nM. These data suggest that homologous up-regulation entails down-regulation followed by an inhibition of receptor degradation.

620.9

MITOGENICALLY-INDUCED ALTERATION OF PERIPHERAL-TYPE BENZODIAZEPINE (τ) RECEPTOR DENSITY ON THE NB2-11C CELL CLONE. J.M. Evans-Shields* and H.E. Laird II. College of Pharmacy, Dep. of Pharmacology & Toxicology, U. of Arizona 85711. The affinity and density of the peripheral-type benzodiazepine (τ) receptors expressed in Nb2-11c cells were compared under different stages of mitogenesis. Previous work from this laboratory (Laird, 17:151, 1989) has shown that Nb2 cells possess a τ receptor and that ligands acting on this site modulate proline stimulated mitogenesis. Therefore, this receptor has a functional role in this system. The Nb2 node lymphomas are unresponsive to GABA receptor function and binding. This might be due to interactions at peripheral benzodiazepine sites on neurons or to indirect effects of compounds such as neurosteroids.

620.10

Hippocampal sympathetic ingrowth alters the kinetics of Phorbol Ester receptor binding. V. Ayyagari and L.E. Harrell. Departments of Neurology and Psychology, VA Medical Center and University of Alabama, Birmingham, AL 35294. Fibers from the superior cervical ganglion (SCG) replace the cholinergic input to the hippocampus following medial septum (MS) lesions. Hippocampal sympathetic ingrowth (HSI) has been shown to induce an upregulation of the carbachol-stimulated PI hydrolysis. We now report 3H-Phorbol-12,13-dibutyrate (PDBu) binding kinetics in the dorsal and ventral hippocampus. Adult male Sprague-Dawley rats were divided into 3 surgical groups: sham surgery; MS lesions; or MS lesions + SCGx. Receptor binding studies were performed 4 to 6 weeks after surgery, using cold PDBu to measure non-specific binding. Preliminary results indicate that MS lesions increase the Bmax, while HSI appears to normalize KD. Both the KD and Bmax of 3H PDBu were consistently higher in the Bmax while HSI appears to normalize KD. These results indicate that depletion of the brain serotonin may antagonize the ability of subchronic administration of IMI to reduce [3H]DPG binding sites in the striatum and hippocampus, suggesting that serotonergic systems could be involved in the regulation of sigma binding sites in these brain areas.
621.1

EFFECT OF SOCIAL STRESS AND HOUSING CONDITIONS ON NEUROENDOCRINE MEASURES
Rockefeller University, 1230 York Ave., NY, NY 10021

Analysis of social and agonistic interactions in rats groups in semi-

natural burrow systems revealed complex patterns of

behavioral changes for subordinate males. These behaviors include

reduced feeding and aggressive activity in sleep cycles,

weight loss associated with lower food intake and other behaviors

concomitant with the outcomes of adrenocorticotropin (ACT)

In the current study we examined 8 groups, each with 45 males

and 2 females in visible burrow systems. Dominant hierarchies were monitored for 2 week colony period, in which a basal blood sample was collected for determination of plasma corticosterone (CORT) levels and the males were adrenalectomized. Brain (hippocampus and cortex), pituitary, spleen, thymus and adrenal glands were removed on the following day. Plasma CORT levels of dominant male rats were lower than those of male subordinates (6.37 μg% vs 16.15 μg%). Adrenal and spleen weights were increased in both subordinates and dominants compared to controls. In contrast, there was no difference between the three groups in Type I or Type II glucocorticoid binding in the brain areas examined.

Data from both subordinates and dominant male rat in a burrow system habitat provide useful insight into the behavioral and neuroendocrine effects of prolonged social stress. (Supported by AA06220/RUI, MH42603/DCB and MH41256/BSM).

621.2

EFFECT OF SWIM STRESS ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS: A TIME COURSE STUDY
Blavatnik Health Research Institute, University of Michigan, Ann Arbor, MI 48109

The anterior pituitary corticotropin uses different biochemical strategies to cope with acute vs chronic stimulation. It has been shown that chronic foot shock stress causes a significant increase in both Proopiomelanocortin (POMC) peptide content and POMC mRNA in the anterior lobe, when compared with acute stress (McEwen et al., 1979). In contrast, chronic swim stress does not cause a significant increase of POMC peptide content in the anterior lobe (Neuroendocrinology 52:405). To further characterize the effects of chronic pituitary-adrenal axis, we performed a time course study in which rats underwent daily swim stress sessions for 1, 3, 7, 30, 14, 17 and 21 days. Rats were sacrificed 24 hours after the last swim session. One group sacrificed immediately after one swim session (acute swim) and one control group were also included. Corticosterone levels were significantly elevated after an acute swim session (ANOVA p < 0.05) but returned to baseline after 24 hours. Corticosterone was again slightly elevated after 3 days of swim and slowly decreased after 7 and 10 days of swim. From days 14 to 21, corticosterone levels began to rise again. Plasma ACTH levels closely resembled corticosterone levels. No statistically significant changes in POMC peptide content or POMC mRNA levels were detected in the anterior lobe at any of the time points studied. The relationship of these findings to changes observed in the intermediate lobe of the pituitary will be presented. Supported by The Robert Wood Johnson Minority Medical Faculty Development Program (J.F.L.) and by MH42225 (H.A., S.J.W).
621.7 MALE AND FEMALE DIFFERENCES IN RESPONSE TO RESTRAINT STRESS IN RATS. W.T. Gallucci*, L. Scheideman,* M.A. Smith, H.L. Whitley and P.W. Gold*. Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Clinical data suggest that stress may precipitate the onset of major psychiatric illness such as depression and eating disorders. Manifestations of these illnesses may be attributed to perturbations in stress-responsive neuroendocrine systems such as the hypothalamic-pituitary-adrenal axis (HPA). Epidemiological studies have shown that these illnesses are much more common in women than in men. Previous studies in animals have shown that female rats are more responsive than male rats to restraint stress and show elevated levels of ACTH, corticosterone and AVP in peripheral blood during a single 2 hour restraint period. In this study, we investigated the secretion of these stress-responsive hormones into the blood after 15, 45, or 180 minutes of restraint stress. After the restraint period, the animals were sacrificed and trunk blood collected. The results indicate that female rats had significantly higher (p<.001) corticosterone levels than male rats over the total restraint period. In addition, female rats had higher peak (p<.002) and basal corticosterone levels (p<.001) than males. The AVP response showed marked differences between the sexes (p<.001). Female AVP levels were elevated after the 15, 45, and 180 minute periods of restraint and showed a 44% increase over males at 45 and 180 minutes. On the other hand, males were not significantly different from baseline at 15, 45 and 180 minutes of restraint. The ACTH response also showed a significant sex difference (p<.005). This response is in contrast to the males whose response is not significantly different from baseline at the 180 minute time period. These findings suggest that female rats produced more ACTH, AVP and corticosterone in response to similar stress conditions. Central factors involving corticosterone-releasing activity may differ between male and female rats which may account for the increased hormonal responsiveness in females. Whether these results are related to behavioral vulnerabilities between the sexes and whether they reflect excess stress system responsiveness of the female sex to stress, remains to be studied.

621.8 EVIDENCE FOR ENHANCED TRANSMISSION OF LYMPHOCYTE GLUCOCORTICOID RECEPTORS IN PTSD. R. Yehuda, E.L. Miller, D. Boisoneau*, S.M. Southwick*, M.T. Lowy and John W. Mason. Psychiatry Department, University of Connecticut Health Center, Farmington, CT 06032.

Our previous finding of a significantly lower mean 24-hr urinary cortisol excretion and a greater number of lymphocyte glucocorticoid receptors (GR) in combat veterans with posttraumatic stress disorder (PTSD) has suggested an enhancive influence of the hypothalamic-pituitary-adrenal axis in this disorder. To further explore this possibility we examined 8:00 a.m. cortisol levels and lymphocyte GR number in 10 PTSD and 10 normal subjects. After baseline cortisol sampling we followed the 11:00 p.m. administration of 0.5 and 0.25 mg doses of dexamethasone (DEX). Consistent with our earlier report, the PTSD group had a significantly larger number of 8:00 a.m. GR at baseline compared to normal controls (mean GR/cell = 8716 ± 1212 and 5128 ± 1284 for PTSD and normals, respectively). Nine hours following DEX, the PTSD group showed a significantly greater decline in cortisol (i.e., a 90% vs. 76% suppression to 0.5 mg DEX, and 58% vs. 30% suppression to 0.25 mg DEX), and a decrease in the number of cytosolic GR (i.e., 40% vs. 14% fewer receptors following 0.5 mg and 19% vs. 3% following .25 mg DEX in PTSD and normals, respectively). Percent cortisol suppression at 8:00 a.m. postDEX was significantly correlated with decreases in GR in both groups at the 0.5 mg dose. The greater decline in cytosolic GR following DEX in the PTSD likely represents an enhanced translocation of the steroid-GR complex into the cell nucleus, and provides further support for HPA dysfunction in this disorder.


Hypothalamus-pituitary-adrenal (HPA) axis responses are characterized by large interindividual differences. The present study was designed to clarify if a specific individual sensitivity of the HPA-axis exists and to which extent it is genetically determined.

Twenty-five monozygotic and dizygotic twin pairs (15-33 yrs) performed physical exercise (ergometry until exhaustion) and were exposed to psychological stress (public speaking and mental arithmetic in front of an audience and a video cameras). Twenty of these twin pairs received an injection of 100 μg h-CRH i.v. HPA-axis activity was indexed by salivary cortisol measures with saliva samples being obtained at 20-min intervals from 8 a.m. to 10 p.m. Moreover, each twin pair spent one day together in their natural environment and saliva samples were collected for analysis of circadian cortisol rhythm at 20-min intervals from 8 a.m. to 10 p.m.

Under all experimental conditions, significant increases in salivary cortisol levels were observed. Analysis of necessity has not been completed results on intra-pair correlations with respect to HPA axis responses in twins will be presented on the meeting.

621.11 CORTICOSTEROSE RESPONSE TO RESTRAINT STRESS IN RATS BRED FOR LEARNED HELPLESSNESS. F.A. Henn, K. Edwards, R.L. Spencer, Y. Katabe and B.S. Mcvicker. SUNY at Stony Brook & Rockefeller University.

We have measured the corticosterone (CORT) response to a 1hr restraint stress of rats bred for learned helplessness (LH). Basal CORT levels in the morning (AM) were lower in LH strain rats compared to rats bred for non-learned helpless (NLH), and basal cort levels of LH rats in the evening (PM) were lower than those of outbred Sprague Dawley controls.

Outbred Sprague Dawley rats, NLH and LH strain rats could be differentiated by their overall CORT response to the restraint stress paradigm. Plasma CORT levels of LH rats immediately after restraint stress was significantly reduced as compared to NLH and outbred controls (μg/100ml: 10.4 ± 1.5, LH vs 32.8 ± 1.7, NLH & 32.1 ± 4.0, outbred controls; n=8/group). Plasma CORT levels of LH rats after a 1hr recovery from restraint stress were lower than NLH in the AM (but not PM) and lower than outbred controls in the PM. There was no significant differences in Type I or Type II steroid receptors in the hippocampus & the hypothalamus of LH strain rats as compared to outbred control and NLH strain rats.

CRF and ACTH mechanisms of LH strain rats are under investigation.


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Early in the development of the hypothalamic-pituitary-adrenal (HPA) axis the rat undergoes a stress hyporesponsive period of blunted responses to several stressors including cold exposure (CE) and maternal deprivation (MD). We examined the development of the axis by monitoring adrenocorticotropic hormone (ACTH) plasma levels in an animal model of depression and/or anxiety characterized by learned helpless (LH) behavior and a dysfunctional HPA axis in adult life (Biol. Psychiatry 26:530, 1989). On postnatal day 7 there was no significant difference between basal plasma ACTH levels among LH rats and controls. On postnatal day 15 there was a 98% increase in ACTH response to CE. On postnatal day 21 baseline ACTH and response to CE were significantly suppressed in LH rats. Stress response to MD was present in all groups but was statistically different for all ages of development between groups. These findings suggest that rats with congenital learned helplessness undergo a differential response in the development of the HPA axis in that the axis was hypersensitive at postnatal day 15 and hyporesponsive by day 21 and this may in part account for the dysfunctional stress response observed during adulthood.


In an attempt to characterize the physiologic, biochemical and cognitive responses to uncontrollable stress, 40 healthy male subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either controllable or uncontrollable stress conditions (UCS). CS subjects could terminate both stressors while their yoked UCS partners could not. Measures of physiologic reactivity, biochemical response, cognitive functioning, and mood were made before and after stress induction. Both CS and UCS stress produced a rise in diastolic and systolic blood pressure during the stress induction period. However, these rises were significantly attenuated in the UCS condition. Several biochemical markers of stress accompanied these physiologic changes and differentiated the UCS from the CS subjects. Cortisol and immune reactive beta endorphin were significantly elevated by UCS. Subjects in the UCS condition also reported higher ratings of helplessness, lack of control, confusion and total mood disturbance. Cognitive performance on a delayed memory task was impaired by both stressors. Results suggest that lack of control over psychological stressors can produce alterations in mood as well as physiologic and neuroendocrine changes in healthy subjects.
Differential Endocrine and Autonomic Regulation of Restraint-Induced HSP70 Expression in the Adrenal Gland and Vascular Muscle

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FRIDAY AM

621.13


Restraint is known to illicit a physiologic stress response characterized by sympathetic nervous system stimulation and activation of the hypothalamic-pituitary-adrenal (HPA) axis. We have shown that restraint induces the selective expression of HSP70 mRNA in the adrenal cortex and blood vessels of the rat and have proposed that their induction in mammalian tissues plays an important role in physiologic stress responses. In this report, we have used perturbations of the HPA axis and sympathoadrenal activity to block the respective contribution of endocrine and nervous pathways to restraint-induced HSP70 expression in vivo. The influence of the HPA axis was investigated by restraining hypophysectomized and control rats for 60 min then determining HSP70 expression in adrenals and aortas by northern blot analysis and in situ hybridization. Hypophysectomy resulted in a 5-fold decline in HSP70 expression in the adrenals but did not significantly alter expression in the aorta. Treatment of normal rats with chronic release pellets containing the glaucocorticoid receptor antagonist RU486 (5 mg) for two weeks prior to restraint also significantly reduced HSP70 expression in the adrenal but had less effect in the aorta. To determine if the reduced responsiveness of the aorta to HPA manipulation was due to alternative regulation in this issue by sympathetic nervous system activity, rats were pretreated with either propranolol (30 mg/kg) or prazosin (1 mg/kg) prior to restraint. Prazosin virtually eliminated aortic HSP70 induction but did not significantly alter its induction in the adrenal. Propranolol was less effective than prazosin in reducing induction in the aorta (5-fold decline) and did not affect induction in the adrenal. Thus, restraint-induced HSP70 mRNA expression in the aorta is modulated by the HPA axis whereas expression in the aorta is controlled by sympathoadrenal activity, each involving distinct receptor mediated events.

PAIN MODULATION: CNS

622.1

AFFERENT PROJECTIONS FROM THE ANTERIOR PRETECTAL NUCLEUS TO THE VENTRAL MEDULLA OBLONGATA: ANTEROGRADE TRACER AND PHASEOLUS VULGARIS LEUCOAGGLUTININ STUDIES. D.R. Onstott and A.J. Beitz, Department of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

Recent electrophysiologic data have shown that the anterior part of the anterior pretectal nucleus (APTA) plays a role in processing nociceptive stimuli while other nuclei of the pretectal complex are engaged in control of visual system reflexes. The ventral medulla oblongata is known to be involved in central cardiovascular and nociceptive regulation and medullo-spinal pathways can transmit descending impulses from several higher centers. In the present study, we made a direct projection from the APTA into the ventral medulla oblongata through anterograde tracer and Phaseolus vulgaris leucoagglutinin (PHA). After injection of Fluoro-Gold into the APTA of rats, retrogradely labeled cells in the ventral medulla were observed in a coronal plane. The number of labeled cells varied from 10 to 20 in each section. Occasionally, these cells were labeled in the same sections using Fluoroprobe 670-labeled secondary antiserum (Jackson ImmunoResearch). Confocal scanning laser microscopy (BioRad MRC-600 Imaging System) was used to produce two separate series of 1 μm "optical sections" of injected cells and of PHA-positive varicosities. Individual images from the two series were stacked and analyzed using a computer program to determine the spatial distribution of labeled cells and varicosities. Fibers descending from the APTA innervated almost exclusively the ipsilateral side of the medulla oblongata. A high density of varicose labeled fibers was observed in the rostral part of the ventral gigantocellular reticular nucleus, in the gigantocellular reticular nucleus pars alpha. Labeled terminals were present in the rostral part of the raphe obscurus and within the raphe magnus. Labeled fibers in the olivary inferior complex were most numerous in the ventral aspects of the dorsal olive. From control injections the majority of labelling in the ventral medulla appeared as non-varicose fibers in the region of the pyramidal tract and the dorsal inferior olive.

A direct APTA-medulla connection might be involved in descending control of nociception.

622.2


Stimulation of vagal afferent nerve fibers has been shown to modulate nociceptive transmission in the spinal cord. It has been proposed that vagal afferent fibers engage medullary raphespinal (RAS) neurons to modulate nociception. The goal of this study was to determine response characteristics of medullary raphespinal (RAS) neurons to electrical and chemical stimulation of vagal afferent fibers. Six cats were anesthetized with sodium pentobarbital (35 mg/kg). Extracellular potentials were recorded from 10 RAS neurons whose axons were antidromically activated from the T2-T3 segments of the spinal cord. Seven cells were inhibited and 3 cells were excited by electrical stimulation of vagal afferent fibers. The inhibitory response to electrical stimulation was intensity and frequency dependent. Left atrial injections of veratridine and phenylbiguanide were used to chemically stimulate vagal afferent fibers. Four of 7 cells inhibited by electrical stimulation were also inhibited by chemical stimulation. Two of 3 cells excited by electrical stimulation were inhibited by chemical stimulation. No cells were excited by chemical stimulation. We conclude that vagal afferent fibers can modulate the activity of RAS neurons and, therefore, can affect RAS influences in the spinal cord. (Supported by NIH grant HL29618 and OCAST grant HR9-069).

622.3


In the CNS, c-fos is an useful marker of neuronal activity that can be used to map functionally related neural pathways. Its expression is enhanced after inflammation in pontine and medullary structures. This study was undertaken to develop a method of quantifying possible functional interactions of c-fos expression with identified interneurons. Our previous studies showed an increase of c-fos gene expression in the spinal cord in response to inflammatory stimuli. The influence of the HPA axis was investigated by restraining hypophysectomized and control rats for 60 min then determining HSP70 expression in adrenals and aortas by northern blot analysis and in situ hybridization. Hypophysectomy resulted in a 5-fold decline in HSP70 expression in the adrenals but did not significantly alter expression in the aorta. Treatment of normal rats with chronic restraint pellets containing the glucocorticoid receptor antagonist RU486 (5 mg) for two weeks prior to restraint also significantly reduced HSP70 expression in the adrenal but had less effect in the aorta. To determine if the reduced responsiveness of the aorta to HPA manipulation was due to alternative regulation in this issue by sympathetic nervous system activity, rats were pretreated with either propranolol (30 mg/kg) or prazosin (1 mg/kg) prior to restraint. Prazosin virtually eliminated aortic HSP70 induction but did not significantly alter its induction in the adrenal. Propranolol was less effective than prazosin in reducing induction in the aorta (5-fold decline) and did not affect induction in the adrenal. Thus, restraint-induced HSP70 mRNA expression in the aorta is modulated by the HPA axis whereas expression in the aorta is controlled by sympathoadrenal activity, each involving distinct receptor mediated events.

622.4

THE RELATIONSHIP OF SUBSTANCE P VARIOSITIES TO PERIAQUEDUCTAL GRAY-RAPIHGLUS MAGNUS PROJECTION NEURONS: ANALYSIS USING INTRACELLULAR INJECTION AND LASER CONFOCAL MICROSCOPY. D.R. Omnell and A.J. Beitz, Department of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

The descending projection from the periaqueductal gray (PAG) to the rostral magnus (RM) has been identified as an important component in the pain modulation system. Substance P (SP) has been shown to produce analgesia when injected into the PAG, and SP-immunoreactive (SP-IR) terminals make synaptic contacts within the PAG. The present study was undertaken to develop a method for quantifying possible sites of interaction between SP-IR varicosities and PAG-RM projection neurons, including sites on distal dendrites not normally filled by retrograde tracers. After injection of Fluoro-Gold into the NRM of rats, retrogradely labeled cells in the PAG were visualized in 150 μm vibratome sections with epifluorescence illumination and injected immunohistochemically with Lucifer yellow (LY). SP-like immunoreactivity was found in the same sections using a confocal laser scanning microscope (BioRad MRC-600 Imaging System) was used to produce two separate series of 1 μm "optical sections" of injected cells and of SP-IR varicosities. Individual images from the same plane in each series were merged to reveal varicosities proper to neuronal elements of LY-filled cells. Complete LY filling of the soma and dendritic tree was accomplished in several cells of varying morphology in each section. Occasionally, dye-coupling between PAG neurons was noted, suggesting the presence of gap junctions. Preliminary examination has indicated that a small number of SP-IR varicosities come into close contact with the soma and the proximal and distal dendrites of PAG-RM projection neurons. This study suggests that retrograde labeling coupled with LY intracellular injection and confocal microscopy may be a useful procedure for elucidating the interactions of peptide-containing varicosities with identified projection neurons. Supported by DA06687, DA06682, DC10806 and NS19208.
622.5 DOMINANCE OF SHT-1A OR SHT-2 AND CHOLINERGIC RESPONSE IN RAPHE MAGNUS CELLS VARIATES WITH PERIPHERAL STIMULATION IN A RAT MODEL OF NOXIOUS STIMULUS. K. Taguchi, M. Anderson*, T. Shibuya, and J. D. Hentati. Univ. of Ill. Coll. of Med., Rockford, IL 61107.

Neurons of the raphe nuclei (NRM) are known to be responsive to serotonin (5HT), possibly via autoreceptors, recurrent axonal branching, or remote inputs. In pentobarbital-anesthetized rats, we examined responses to SHT, serotonin agonists, buspirone, to SHT-1A antagonists propranolol and methiothepia, to SHT-2 antagonist ketanserin, to mttigated antiserotonin, and to acetylcholine (ACH). Neuronal responses to skin pinching were also tested. As well as previously known on-cells and off-cells, we found at ventral sites neurons with biphasic responses to prolonged (10s) pinching: the excitatory and inhibitory responses became predominant, respectively, at low and high synaptic activity.

622.6 LAMINA II CELLS PROJECT STRONGLY TO THE VENTROLATERAL RETICULAR FORMATION OF THE MEULLA OBLOGNATA IN THE RAT E. Lima*, A. Vaz, and H. L. Fields. (Society for Neuroscience Association) Institute of Histology and Embryology of the Faculty of Medicine of Oporto, 4200 Porto, Portugal.

The substantia gelatinosa laminae III and IV of the rat spinal cord is considered a pain modulatory system acting locally upon the transmission of nociceptive input through dorsal horn neurons. Several studies using anterograde tracing strategies showed no significant numbers of lamina I cells projecting supraspinally. Stereotaxic injections of 1.5% cholera toxin subunit B (CTB) in the ventrolateral reticular formation intermediate between the lateral reticular nucleus and the spinal trigeminal nucleus, pars caudalis, of adult, chloralose anesthetized rats, resulted in retrograde labelling of spinal neurons in laminae I-III and the lateral spinal nucleus. Labelled lamina II cells were on average 430 in the cervical and 210 in the lumbar enlargement, representing, respectively, 28% and 21% of the labelled spinal neurons. Most cells had dendritic arbors spread in the parasagittal plane making up a circular dendritic field across lamina II. The ventrolateral reticular formation thus appears to be a specific target of lamina II cells. It is suggested that these neurons exert a modulatory action upon the descending, probably noradrenergic antinociceptive input generated in that modulatory region. (Supported by SNF, project no P9CT-CSHA/31690)


The physiological and pharmacological properties of two classes of putative nociceptive modulatory neurons in the rostral ventromedullary medulla (RVM) have been extensively characterized in rats lightly anesthetized with barbiturates. "On-cells" show a transient increase, and "off-cells" a sudden decrease immediately preceding nociceptive reflexes. We have recently shown that the reflex-related changes in neuronal activity in the RVM are gated by barbiturates are known to potentiate GABAergic influences. It is important to demonstrate that these neurons can be characterized in non-barbiturate preparations.

Activity of RVM neurons was recorded in rats maintained under halothane anesthesia (0.5-1.0% in oxygen, 0.3 l/min). Pain-related stimuli that increased an increase in activity (on-cells) or a decrease in activity (off-cells) that immediately preceded the occurrence of the tail flick were found in this preparation. On-cells were found more frequently, but this seemed to result at least in part from using somatic stimulation as a search stimulus for cells of this class. When noxious inputs were limited by testing only one cell per animal, similar numbers of on- and off-cells were found.

Thus, both on- and off-cells can be identified in rats anesthetized with halothane, indicating that neurons with these properties are not an epiphenomenon of barbiturate anesthesia. Moreover, the reported inability to demonstrate off-cells in the awake rat (Oliveras et al., 1989; 1990) may be due to the lack of an appropriate search stimulus for cells of this class.

Supported by PHS grants DA01949 and DA05608 and a Pain Research Grant from the Bristol-Myers Squibb Company. MWM was supported by DA05399.

622.8 NOCEPTIVE MODULATORY NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA ARE FOUND IN BOTH HALOTHANE- AND BARBITURATE-ANESTHETIZED RATS. M. Morgan, M. Heinricher and H. L. Fields. Dept. of Neurology and Physiology, Univ. of California, San Francisco, CA 94143.


A confocal microscopic study of enkephalin immunoreactive appositions onto physiologically identified RVM neurons of different physiological classes. The present study, therefore, investigates the distribution of enkephalin-immunoreactive appositions onto physiologically identified RVM neurons. RVM neurons were characterized by their response during a nociceptive withdrawal reflex in lightly anesthetized rats. Cells were then injected intracereally with Neurobiotin and subsequently visualized with a Texas Red fluorophore. Sections that contained labelled neurons were processed for enkephalin immunoreactivity using a FITC fluorophore. These sections were optically sectioned at 1 milimeters using a dual channel confocal laser scanning microscope (MRC-1024), and preselected between immunoactive varicosities and intracellularly labeled neurons were mapped onto reconstructions of on-, off-, and neutral cells. Enkephalin appositions were found on the somata and dendrites of all on-cells. Although enkephalin varicosities were also apposed to some off-cells, their density was significantly less than that on on-cells. These results support the idea that on-cells are predominantly inhibited by opioid peptides and that the opioid excitation of off-cells is produced indirectly, possibly by opioid blockade of an on-cell mediated inhibition.

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622.17

NEURONAL RESPONSES IN CINGULATE CORTEX TO NOXIOUS STIMULATION MAY ORIGINATE IN MEDIAL THALAMUS

Anterior cingulate cortex has been implicated in affective responses to noxious stimuli. This study investigates the neural basis for such responses. Single-unit recordings were made in area 24 of anesthetized, tail-belted rabbits anesthetized with halothane. Stimuli included transcutaneous electrical, mechanical and thermal stimulation. About 25% of cingulate neurons responded to noxious levels of these stimuli and did not respond to innocuous levels. Nearly 54% of the units that responded to noxious stimulation were located in layers II and III. Excitatory units were located mainly in layer III while most inhibitory units were in layer II. Only about 16% of layer V units responded to these stimuli.

To identify the source of this input, cortical lesions were made separating area 24 from adjacent cortical areas. In other animals, lidocaine injections were made to reversibly block neuronal activity in the medial thalamus. Responses to noxious stimuli occurred after complete cortical lesions while lidocaine injections reversibly blocked the response of all units.

In conclusion, neurons in cingulate cortex respond to noxious stimulation and this signal may originate in the medial thalamus.

622.18

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Recent studies indicate the existence of CNS input from the uterus and other pelvic organs which gives rise to conscious sensations, including pain, suggesting that neurons in somatosensory relay nuclei receive such input (1). The present study examined responses of 61 neurons in and near the thalamic ventrobasal nucleus (VB) of 14 estrous female rats anesthetized with nitrous oxide/halothane to mechanical stimulation of uterus, cervix, vagina, colon and somatic structures (skin, joints). Neurons responsive to uterus (20%) were located throughout VB, preferentially dorsally and rostrally (as were neurons responsive to colon stimuli : 18%). Of 42 viscero-responsive neurons, half also had somatic receptive fields, mostly on the hindlimb (86%), but also on forelimb or face (14%). Many (62%) required noxious levels of somatic stimulation. While these neurons (38% of sample) were located equally within VB and its dorsal or ventral borders, neurons responsive only to somatic stimulation (30% of sample) were located primarily within VB and those responsive only to visceral stimulation (28%) were all localized in VB dorsal (88%) or ventral (12%) borders. These results reveal a surprisingly large, well-organized representation of internal female reproductive organs in and near VB.

(Supported by MRT-France and R01 NS11982-NIH, USA.)
(1) Berkley et al., 1988; 1990a;1990b; Berkley, 1990.

PAIN MODULATION: MONOAMINES

623.1

RECEPTOR MEDIATION OF 5-HT-INDUCED INFLAMMATORY NOCICEPTION IN RATS.
K.I. Sufta, F. Schomburg* and I. Giordano.
Neuropharmacology Lab., COPS, Drake Univ., Des Moines, IA 50311.

The present study examined the effects of intraplantar (ipl) injection of 5-HT and the ability of selective 5-HT receptor antagonists to differentially attenuate 5-HT-induced edema and nociception. Ipl 5-HT (0.05-1 mu mol) produced dose-dependent hindpaw edema and nociceptive responses (lifts/licks of affected limb). The 5-HT1 antagonist, methysergide (MS: 1-10 nmol; ipl); 5-HT2 antagonist, ketanserin (KT:30-300 nmol; ipl) and 5-HT3 antagonist, odanaceton (OD:100 nmol - 1 mu mol; ipl) differentially affected the nociceptive and edematous response produced by a fixed algesic dose of 5-HT (0.25 mu mol; ipl). Ranked by magnitude of inhibition of 5-HT-induced nociception, MS > KT > OD. These results demonstrate the differential involvement of heterogeneous 5-HT receptors in 5-HT-induced inflammatory nociception.

623.2

PATTERNS OF ANALGESIA PRODUCED BY S AND R ISOMERS OF THE NOVEL 5-HT3 RECEPTOR ANTAGONISTS ADR-851 AND ADR-882 IN RATS.
I. Giordano and K.I. Sufta.
Neuropharmacology Lab., COPS, Drake Univ., Des Moines, IA 50311.

The present study examined the antalgic efficacy of S and R isomers (0.1-10 mg/kg; sc) of the novel 5-HT3 receptor antagonists, ADR-851 and ADR-882 against acute thermal, mechanical and formalin-induced inflammatory pain in male rats. No motoric, overt behavioral or physiologic effects were produced by any dose of S and R isomers of ADR-851 and ADR-882. Neither isomer of ADR-851 or ADR-882 was analgesic in the thermal or mechanical pain test. In the formalin test, ADR-851 produced significant antalgic effects at 3 mg/kg and 10 mg/kg doses. ADR-851S produced analgesia only at 1 mg/kg. Neither isomer of ADR-882 was effective in this assay. These results suggest that by antagonizing peripheral 5-HT3 receptors, ADR-851 may be useful in the therapy of inflammatory pain.

623.3

EFFECTS OF THREE ALPHA-2 ADRENERGIC AGONISTS GIVEN INTRATHECALLY IN RATS.
P.R. Douglass, E. Carstens and F.J. Pascoe*, Depts. of Animal Physiology and Veterinary Surgery, University of California, Davis, CA 95616.

Systemically given alpha-2 adrenergic agonists can produce analgesia, marked sedation and significant cardiovascular changes. Intrathecal (IT) administration produced more intense analgesia with fewer systemic effects. This study examined the analgesic and sedative properties of 3 alpha-2 agonists given IT.

Forty male Sprague Dawley rats had IT catheters placed via the cisterna magna with the tip lying at the thoracolumbar junction. At least 2 weeks after surgery rats were randomly assigned to one of 4 groups to receive 10 ul IT injections of saline, xylazine (30,60,120 ug), detomidine (DET: 20,60,100 ug) or dexmedetomidine (DEX; 2,5.5,10 ug). Drug dose order was randomized. Tail flick latency and motor function were assessed 5,15,30,45,60,90 and 120 min following IT drug injection.

IT DET and DET produced significant, dose-dependent analgesia. Sedation accompanied analgesia at higher doses but was usually of shorter duration. Analgesia and sedation were prevented by pre-administration, and reversed by post-administration, of the alpha-2 antagonist atipamezole (1 mg/kg). Xylazine produced analgesia and sedation at higher doses with antagonism by atipamezole.

Thus, appropriate doses of DET or DET may afford analgesia with less accompanying sedation.

623.4

DIFFERENTIAL EFFECTS OF MONOAMINE UPTAKE BLOCKADE ON MU, KAPPA, AND KAPPA3 ANTAGONISTS. D. Paul.
Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Selective stimulation of mu, kappa, or kappa opioid receptors produces analgesia through neuroanatomically and neurophysiologically distinct mechanisms. Blockade of monoamine uptake potentiates morphine analgesia. To determine whether monoamine uptake blockade will potentiate kappa, and kappa, analgesia, as well as mu analgesia, we assessed the analgesic effects of USO, 4887, H110Z0H and morphine in mice treated with clomipramine or saline using the tail-flick assay. We first determined that 1 mg/kg of clomipramine, given 2 hr before testing, produced a 10-fold shift to the left of the analgesic dose-response curve for intrathecal ME without affecting intrathecal 5-HT analgesia. This dose by itself did not produce analgesia. The clomipramine treatment potentiated the analgesic effects of morphine (10 mg/kg s.c.), but had no significant effect on USO, 4887 analgesia (2 mg/kg, s.c.). These results indicate that a monoamine may modulate kappa, analgesia as well as mu analgesia.
623.5 ENEKPHALINERGIC ANTIINOCICEPTION INDUCED BY ELECTRICAL STIMULATION AND SUBSTANCE P MICROINJECTION INTO THE A7 NORADRENERGIC CELL GROUP. D. C. Yeomans and H. K. Proudfit. Dept. of Pharmacology, Univ. of Ill. at Chicago, Chicago, Ill. 60612.

We have recently demonstrated that the A7 catecholamine cell group in Sprague-Dawley rats (Baxo, Inc.) provides a dense noradrenergic innervation of the dorsal horn of the spinal cord. We have also demonstrated that activation of A7 cells by either electrical stimulation or microinjection of Substance P induces antinociception which can be attenuated by intrathecal injections of nonselective α-adrenergic antagonists. In addition, we have used neuroanatomical tract-tracers combined with immunocytochemistry, to show that some met-enkephalin immunoreactive cells in the A7 area project to and terminate in the dorsal horn. To investigate the possibility that cells in the A7 might be involved in the antinociception induced by stimulation of the A7 area, we determined whether such antinociception could be blocked by intrathecal injection of an opioid antagonist using lethally anesthetized (urethane 1.0 g/kg) rats. The latency to foot withdrawal in response to a strong thermal stimulus was used as a measure of nociception. Electrical stimulation of the A7 area induced antinociception that was reduced by intrathecal injection of the opioid antagonist naltrindole (30 μg in 15 μl saline). Similarly, microinjection of 0.7 μg of Substance P into the A7 area produced antinociception which was also partially antagonized by intrathecal administration of the same dose of naltrindole. These results support the proposal that spinally-projecting enkephalin-containing neurons near the A7 cell group can modulate nociception. Supported by USPHS Grants DA03980 to HKP and DA04508 to DCY.

623.6 EVIDENCE THAT THE ANTIINOCICEPTION PRODUCED BY STIMULATION OF NEURONS IN THE DORSOLATERAL PONTINE TEGMENTUM IS MEDIATED BY RAPHÉ-SPIINAL SEROTONIN NEURONS. L.A. Proudfit. Department of Pharmacology, University of Illinois at Chicago, Chicago, Ill. 60680.

Anatomic evidence indicates that neurons originating in the dorsolateral pontine tegmentum (DLPT) project to the nucleus raphe magnus (RMG). These neurons may contribute to the modulation of nociception by activating the spinally projecting serotonin neurons in the NRM. To test this hypothesis, the DLPT, specifically the region near the A7 noradrenergic cell group was stimulated in partially unanesthetized female Sprague-Dawley rats. The latency to elicit a reflex paw-withdrawal to noxious heat was determined before, during and after electrical stimulation. Stimulation of the A7 region resulted in antinociception as measured by increased paw-withdrawal latencies. This effect was blocked by the intrathecal administration of the serotonin antagonists methysergide and propranolol, but not by saline. These findings provide evidence for the existence of projections from the DLPT to the RMG, which are involved in modulating nociception. (Supported by USPHS grant DA03980.)


We have shown that noradrenergic neurons in the A7 catecholamine cell group innervate the spinal cord dorsal horn in rats. This observation suggests that these neurons may modulate nociception by inhibiting nociceptive transmission in the spinal cord. This hypothesis was tested by determining whether chemical stimulation at various sites in the dorsolateral pontine tegmentum in lightly anesthetized rats could modify nociception. The results indicated that stimulation at sites near the A7 cell group produced antinociception that was reduced by intrathecal injection of the α2-receptor antagonist phenolamine and yohimbine. The results of these experiments provide evidence that pontospinal noradrenergic neurons located in the A7 cell group are important components of the descending neuronal system that inhibits nociception. Supported by USPHS Grant DA03980.


Previous studies provide evidence for the existence of α1- and α2-adrenergic receptors in the ventromedial medulla that are involved in the modulation of nociception. The present studies characterized the adrenoceptor subtypes in this area using radioligand binding assays. α1 receptors were characterized using [3H]prazosin. Ligand analysis yielded a Kd of 89 pM and a Bmax of 48 femtomoles/mg protein. Displacement studies were performed using WB4101 and phenolamine. Ligand analysis of these data best fit a one-site model. Treatment with chlorotetralin chloride, which selectively allows α2 receptors, reduced specific binding by 70 to 80%. These data suggest that [3H]prazosin binds to a single site with characteristics of the α1p receptor. α2 receptors were studied using [3H]rauwolscine. Ligand analysis yielded a Kd of 16 nM and a Bmax of 53 femtomoles/mg protein. Displacement studies were done using prazosin, yohimbine, and idazoxan; these data were best fit by a one-site model. The Kd values were 1.5 nM, 200 nM, and 140 nM, respectively. The high affinity of prazosin and the low affinities for yohimbine and idazoxan suggest that the majority of the radioligand binding sites appear to correspond to the α2b subtype. Supported by USPHS Grant DA03980.

623.9 RELATIVE INTRINSIC ACTIVITY OF INTRATHECALLY ADMINISTERED ALPHA 2 AGONISTS, DEXMDETOMIDINE AND CLONIDINE, AS DETERMINED BY THE USE OF EEDQ, AN IRREVERSIBLE ANTAGONIST Y. TAKANO, M. GRAFE & L. YAKSH. Department of Anesthesiology, University of California, San Diego, La Jolla, CA 92039.

Intrathecal dexmedetomidine and clonidine produced a dose dependent antinociception, which, based on the similar relative antagonist potency of intrathecal α2-adrenergic antagonists (atipamezole = idazoxan >> yohimbine) prazosin) suggests that these agents have α2-adrenergic receptor site. To examine the relative intrinsic activity of these spinalally administered α2-adrenergic agonists in vivo, dose response curves for dexmedetomidine and clonidine were carried out on the 52°C hot plate test with Sprague-Dawley rats using chronically implanted intrathecal catheter. EEDQ (N-ethylcyclohexyl-2-ethoxy-1,2-dihydroquinoline), an irreversible α2-adrenergic antagonist was administered in dose of 8.1, 81 or 810 nmol, 24 hours before the spinal administration of one of the two agonists. Each animal was used once with a single dose of a single agonist. EEDQ alone induced in an acute mild hyperalgesic effect. Intrathecally injected dexmedetomidine and clonidine resulted in a dose dependent increase in the hot plate response latency. ED50 of the percent maximal possible effect (%MPE) = 10 (7.3-15) nmol and 118 (86-164) nmol, respectively. Pretreatment with intrathecal EEDQ caused a rightward shift of dose-response curve and reduction of the maximal effect of both dexmedetomidine and clonidine. Analysis of double reciprocal plot indicated that the fractional receptor occupancy required to produce the EEDQ was 10 % and 38 % for dexmedetomidine and clonidine, respectively. These results suggest that dexmedetomidine has a higher intrinsic activity than clonidine to produce an antinociceptive response when administered intrathecally. These experiments were supported partly by Dokkyo University, School of Medicine(YT) and DA02110(TL).
623.12

THE ROLE OF NOREPINEPHRINE IN DAMPGO-INDUCED ANTI-

*NOTE: This abstract is a duplicate and should not be considered.

623.13

THE EFFECT OF 5-HT1A AGONISTS, SEROTONIN AND RAPHE

STIMULATION ON SPINAL DORSAL HORN NOCICEPTIVE

NEURONS IN THE RAT. R. Maureen Murphy, M.M. Behbehani,

A.Z. Murphy, and F.P. Zemlan. Depts. of Psychiatry and Physiology,

Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0559.

The effect of iontophoretic application of serotonin (5-HT),

5-HT1A agonist iontophoresis on the same dorsal horn cells revealed that

3-HT stimulation significantly inhibited 48% of the 78 units tested while

13% were excited. Iontophoretic 5-HT administration inhibited 10 of

15 cells also inhibited by NRM stimulation while iontophoretic administra-

41% of these cells were classified as either nocispecific or nocicompartmentalized.

Kynurenic acid was effective in blocking the excitatory effects of HA-induced

The majority of these cells were classified as either nocispecific or

wild-type dynamic range. Of the cells that were inhibited by peripheral

stimulation, 56% were also inhibited by NRM stimulation. Response to

1 Hr single pulse stimulation showed that 24/50 cells were

responsive: 75% were excited and 25% were inhibited. A post-

excitation inhibitory response indicative of a collateral inhibition

circuitry was noted in 33% of the cells. Phenotypic differences

in blocking this response suggesting the involvement of norepinephrine in this system.

Supported by PHS grant NS20643.

623.14

THE ANTIINOCICEPTIVE EFFECTS OF I.V. CLONIDINE ARE

ENHANCED IN ARTHRITIC COMPARED TO NORMAL RATS

V. Kayser*, G. Guilbaud and J.M. Besson U 161 INSERM, 2, rue

d'Alesia, 75014 Paris, France.

The antinociceptive effects produced by an acute i.v. injection of the

prototypic alpha-2 (α2)-adrenergic agonist clonidine (0.03-0.1

mg/kg) were investigated in normal and in Freund’s adjuvant-induced

arthritic rats used as a model of clinical pain. As a nocicase test, we

used the measure of the vocalization threshold (VT) elicited by

foot pressure. Antagonism of clonidine’s effects by the α-2

adrenoceptor antagonist yohimbine and idazoxan (0.25-1 mg/kg i.v.,

administered 30 min before clonidine) was also tested.

In normal rats, clonidine produced significant dose-dependent effects (mean VT increased by 24, 32 and 35 % of the control at 10

min after the injection, respectively for 0.03, 0.05 and 0.1 mg/kg i.v.

clonidine). An enhancement of the antinociceptive effect of clonidine (0.1, 0.3 and 1 mg/kg i.v.) in these animals.

In arthritic rats, clonidine displayed a more potent and prolonged

antinociceptive effect than in normal rats (mean VT increased by 60, 63 and 86 % of the control, respectively for 0.03, 0.05 and 0.1 mg/kg i.v.

clonidine). For the highest dose used (0.1 mg/kg), the antinociceptive effect of clonidine lasted for up to 110 min in these animals.

In both groups of rats, yohimbine or idazoxan (0.25 to 1 mg/kg i.v.) significantly antagonized clonidine-induced antinociception in a dose-dependent manner.

These results suggest that α-2 adrenoceptors are involved in

clonidine-induced antinociception in both normal rats and this model of inflammatory pain.

Spectrin is a major structural component of the mammalian central nervous system and is localized to synaptic and non-synaptic plasma membranes. The presence of spectrin in the visual system has been previously reported, but the distribution and function of spectrin in the retina is not well defined. To address this, we have used a combination of immunohistochemistry and biochemical fractionation to characterize the localization of spectrin in the retina. Immunohistochemical analysis using anti-spectrin antibodies revealed that spectrin is present in the inner and outer nuclear layers of the retina. Biochemical fractionation showed that spectrin is enriched in synaptosomal fractions, indicating a synaptic localization. These findings suggest that spectrin plays a role in synaptic function in the retina.

624.2 THE ISOLOCALIZATION OF DIRADYL CHOLINE PHOSPHOLIPID, INCLUDING THE PRECURSOR OF PLATELET-ACTIVATING FACTOR, FROM RETINA. L.S. Hurst, and M.G. Bazan. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.

Choline phospholipids (PC) constitute an important source of both arachidonic acid (the precursor of PGH2, TXA2, and prostacyclins) and diacylglycerol (DAG, the precursor of platelet-activating factor (PAF), which are important mediators in many tissues. We have established that choline phospholipids are enriched in the retina, and that some are preferentially localized in a synaptic vesicle fraction. These findings suggest that choline phospholipids play a role in synaptic function in the retina.
**624.7**


The present study investigated co-localization of α1BP-LI, α2BP-LI and nAChR-LI. The retinal sections were incubated with antisense RNA probes. The specificity of the probes was checked by hybridization with sense RNA probes.

**624.8**


The present study was undertaken to investigate the distribution of GABA transporter in the rat retina. The sections were incubated with antisense RNA probes and processed for autoradiography. The results suggest that GABA transporter is expressed in the photoreceptor cell layer and the ganglion cell layer.

**624.9**

**EXPRESSION OF GABA NEURONAL TRANSPORTER mRNA IN THE RAT RETINA.** Christline Weigmann* and Nicholas Brecha. Deps. of Anatomy & Cell Biology and Medicine, Jules Stein Eye Institute and CURE, UCLA School of Medicine and VAMC-Wadsworth, Los Angeles, CA.

The present study investigated the expression of GABA transporter mRNA in the rat retina. The sections were incubated with antisense RNA probes and processed for autoradiography. The results suggest that GABA transporter is expressed in the photoreceptor cell layer and the ganglion cell layer.

**624.10**

**GABA_3 RECEPTOR FUNCTION IN RETINAL NEURONS: MODULATION BY CYCLIC AMP.** M. L. Vergara and H. H. Yeh. Neuroscience Program, University of Rochester Medical Center, Rochester, NY 14642.

The present study was undertaken to investigate the role of GABA_3 receptor function in retinal neurons. The results suggest that GABA_3 receptor function is modulated by cyclic AMP.

**624.11**

**EFFECTS OF DOPAMINE D_1 AND D_2 RECEPTOR AGONISTS ON ELECTRICAL COUPLING BETWEEN FISH HORIZONTAL CELLS.** Kristine Harranyi* and Stuart C. Mangels. Dep. of Ophthalmology, Univ. of Alabama School of Medicine, Birmingham, AL 35294.

The present study investigated the effects of dopamine receptor agonists on electrical coupling between fish horizontal cells. The results suggest that dopamine receptor agonists can modulate the electrical coupling between horizontal cells.
624.13
TYROSINE DEPENDENCE OF DOPAMINE RELEASE IN STIMULATED RETINA. C.J. Gibson. Dept of Pathology, University of Western Ontario, London, Canada N6A 5C1.

Retinas were removed from male Sprague-Dawley rats and placed in individual perfusion chambers for the measurement of endogenous dopamine (DA) release (Gibson, J. Neurosci.Methods, 12: 75-79, 1989). The retina was at least 5 to 7-fold above basal release following a 10 minute pulse of 40 mM potassium (K). In this system, perfusion with a second pulse of 100 μM K resulted in a roughly similar increase in releasable DA. Expressed as a ratio of the fractional release, K2/K1 = 0.96 ± 0.07. When 100 μM tyrosine was added throughout the perfusion this ratio increased to 1.4 ± 0.2 (p<0.05). Addition of a-methyl-p-tyrosine (50 to 200 μM) progressively decreased the amount of DA released. At 200 μM, DA release during the second K pulse was totally abolished. In this system, in which DA release is dependent on newly synthesized transmitter, the addition of the precursor tyrosine maintains and even increases the amount of DA released. (Supported by the Canadian MRC).

624.14
MELATONIN RECEPTOR-MEDIATED INHIBITION OF CYCLIC AMP ACCUMULATION IN CHICK RETINAL CELL CULTURES. P.M. Iuvone and G.J. Gibson. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

In addition to its role as a pineal hormone, melatonin is synthesized in vertebrate retina, where it acts as a signal in photoreceptors. A recent human study has implicated in the circadian regulation of photoreceptor outer segment disk shedding and dark-adaptation, and in the regulation of dopamine release from retinal amacrine cells. The molecular transduction mechanism(s) responsible for melatonin’s actions in retina are not known. We have examined the effect of melatonin and related indoles on cyclic AMP accumulation in glia-free monolayer cultures of photoreceptors and neurons prepared from embryonic chick retinas. Melatonin had no significant effect on basal cyclic AMP accumulation, but significantly inhibited forskolin-stimulated cyclic AMP formation. Melatonin inhibited cyclic AMP formation in both photoreceptor- and neuron-rich cultures, suggesting that both photoreceptors and multipolar neurons can contain melatonin receptors. Melatonin receptor agonists and related indoles inhibited cyclic AMP accumulation with an order of potency indicative of action at a melatonin receptor: 2-iodomelatonin > melatonin > 6-chloromelatonin > 6-hydroxymelatonin > 5-methoxytryptamine > 6-acetyltryptamine > serotonin. Melatonin was potent, acting in the picomolar - nanomolar range, and produced a maximal inhibition of approximately 60% of the cyclic AMP accumulation evoked by 1 μM forskolin. The effect of melatonin on cyclic AMP formation was inhibited by the putative cyclic AMP receptor antagonists luzindole (N-0774) and N-acetyltryptamine. Inhibition of cyclic AMP formation by melatonin was blocked by pretreating the cells with pertussis toxin. The results suggest that melatonin’s actions in the retina may involve receptor-mediated inhibition of cyclic AMP formation. Supported by NIH ROI-1-EY04864.

624.15
TISSUE-TYPIC CELL PRODUCTION IN RETINAL SLICE CULTURE. Andreas F. Mack*, Thomas L. Kasten* and Russell D. Fernand. Institute of Neurochemistry, University of Oregon, Eugene OR, 97403 and Department of Psychology, Stanford University, Stanford, CA 94305.

Two features distinguish retinas of teleost fish from those of warm blooded vertebrates: 1. Teleost retinas can regenerate after lesioning and perfusion this ratio increased to 1.42 (p<0.05) and 2. They continue to grow after embryogenesis, generating new retinal processes in the African cichlid fish Haplochromis burtoni. We used 3H-thymidine to label newly divided cells and immunocytochemistry with rod and cone specific antibodies to demonstrate differentiation of proliferating cells in culture. After 2-3 days in culture, double labelled cells were distributed in a tissue-typic pattern. Newly generated cells were found at the margin and in the outer nuclear layer (ONL). Those in the ONL were labelled with a rod specific antibody whereas those near the margin were principally labelled with a cone specific antibody. This demonstrates that tissue-typic division and differentiation continue in the slice preparation. However, some presumptive rod progenitor cells central from the proliferating edge of the retina also stained positively with the cone antibody. This suggests that rod progenitors are possibly multipotent and may be able to differentiate into photoreceptors other than rods. For example, rod progenitor cells might get important cues for differentiation from their cellular environment and their alteration could play a role in changing their fate during retinal regeneration. We are now analyzing candidate factors which play a role in proliferation and differentiation of the retinal slice.

Supported by EY 00501.

624.16
CHEMICAL AND ELECTRICAL SYNAPSES FORMED BY CHICK RETINAL NEURONS MAINTAINED IN DISSOCIATED CELL CULTURE. E. Gibson, G. Wilson. Department of Zoology, University of California, Davis 95616.

Sparsely cultured retinal neurons derived from day 8 chick embryos form synapses that may be identified ultrastructurally and whose physiology we have begun to investigate. Communication between 103 pairs of large (cell bodies 12-20 μm diameter) multipolar cells has been examined using the perforated patch technique to voltage clamp both neurons. Electrical coupling was present in 61% of the cell pairs and was always ohmic, time-independent and ranged in magnitude from 100 pS to several nS. Overall, chemical synaptic communication was seen in 55% of cell pairs with 49% of these pairs showing reciprocal connections. The frequency with which chemical synapses were observed increased over time in culture. On day 5 in culture 44% of pairs showed chemical synapses whereas on day 9, 73% of cell pairs showed chemical communication. Presynaptic depolarization positive to -30 mV elicited postsynaptic currents that were always noisy and could often be resolved into discrete events with conductance values as large as 1 nS. Spontaneous discrete events of similar amplitudes could also be seen in 28% of cell pairs. Discrete events peaked within 13 msec, decayed with time constants of about 18 msec, and were reversibly abolished by 3 mM Co++. All postsynaptic currents were outward positive to -60 mV and in all cell pairs examined (n=7), 3-10 μM bicuculline reversibly inhibited the current, suggesting that GABA was the transmitter at these synapses. This work was supported by NIH EYO4012.

624.17
ORGANOTYPIC SLICE CULTURE OF THE MAMMALIAN RETINA. W. Wässle and A. Feigenspan*. Max-Planck-Institut für Hirnforschung, Frankfurt, Germany.

Vertical slices of 6 day postnatal rat retinas were cut at a thickness of 100 μm and cultured using the roller culture technique of Gähwiler (J. Neurosci.Meth., 4: 329-342). After 14-21 days in vitro, the slices showed the typical pattern of layering of mature retina. The following immunocytochemical markers were used to characterize the different cell types: antibodies against protein kinase C (PKC), calcium binding protein (CaBP 28 kD), neurofilaments (NF), glial specific antibodies (GFAP, vimentin) and transmitterspecific antibodies (GABA, TH). These markers labelled populations of cells comparable to those in the mature mammalian retina. In particular, antibodies against PKC in the mature retina stain rod bipolar cells. In 6 day postnatal retina no staining was observed. However, after 14-21 days in the slice culture many presumptive rod bipolar cells were labelled. The experiments show that a mammalian slice culture can be used to study differentiation of retinal cell types.

Supported by NIH RO1-EY04864.

624.18
VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN NORMAL AND DIABETIC HUMAN RETINA. E.D. McGookin1*, E.G. Stoop1, R. Cherkv1, G.J. Collins1, A. Buck2, A.M. Gonzalez2, A. Hanneken2, and J. Chorsky1*. Pathology and Ophthalmology SUNY-HSC Syracuse, New York 13210, The Whititer Institute for Diabetes and Endocrinology, LaJolla, California 92037.

The recent identification of vascular endothelial growth factor (VEGF) has indicated this growth factor is a mitogen for vascular endothelial cells. Studies, using immunocytochemistry, compared the distribution of VEGF-like and basic fibroblast growth factor-like (bFGF) immunoreactivity in human eyes, snap frozen post-mortem from control and diabetic subjects. Ten micron sections were cut and stained with specific polyclonal antibodies directed against the amino terminus of VEGF (Ab#1) and bFGF (Ab#773) respectively. The results indicate that bFGF-like immunoreactivity was present in the ganglion, inner nuclear and outer nuclear cell layers of the retina. VEGF-like immunoreactivity was most noted in the ganglion cell layer and in contrast to bFGF, VEGF was more diffusely distributed within the inner nuclear and outer nuclear layers. In addition to their presence in neurons, both bFGF and VEGF were also seen associated with the neovascularization of diabetic retinopathy. Although VEGF is thought to have a role in angiogenesis, our data provide evidence that VEGF may also be an important growth factor in the retina and central nervous system. Supported by AG 09301, NS 28121.
624.21

The pathogenesis of epiretinal membranes (ERMs), which can lead to traction retinal detachments (TRDs), is not clearly understood. To induce ERM formation, human RPE cells were injected into rabbit vitreous and the eyes were enucleated grossly, ultrastructurally, and immunocytochemically at 7, 14, 16, 21, and 28 days. Early TRDs were seen at 7 days and progressed thereafter. Donor RPE cells stopped expressing keratin (K) intravitreally as in vitro cultures. By 21 days, some K+ cells were evident and some RPE cells appeared to begin re-acquiring pigment.

624.22

The effect of the high intravitreal pressure on artificial glaucoma was investigated in 20 albino rats. Isotonic saline was perfused into the anterior chamber of the right eye, as ischemia/reperfusion (I/R) eye, with intracorcular arterial perfusion pressure (PP) for 1 hr (ischemia), then decreased the intracorcular pressure to be normal for another hr. The left eye, as simple ischemia (SI) eye, was just perfused only with the PP for 1 hr (reperfusion). As comparing these two groups, the malondialdehyde in I/R retina was higher than that in SI retina (p<0.001). When both of the I/R and SI retinas were incubated in 1% lanthanum nitrate solution, a lot of lanthanum grains were seen inside along the cytoplasmic membrane of the retinal ganglion cells (RGCs) in I/R group, but the grains were accumulated outside the RGCs in SI group. Morphologically, the clumped nuclear chromatin, vacuolization of the cytoplasm in the RGCs could be observed under light microscope. By electron microscope, the thornobism in the capillaries and the swollen endothelial cells were demonstrated obviously. The changes of the RGCs included increase in electron density, the disruption of cytoplasmic membrane and/or nuclear membrane, the changes of the RGCs included increase in electron density, the disruption of cytoplasmic membrane and/or nuclear membrane, the swelling and vacuolation of mitochondria (Mi). In constrast, only a little changes such as the mild dilatation and the cristae disappearance of the Mi were recognized in the RGCs of the SI retina. These results suggest that the virtual injury occurred mostly during the postischemia rather than the ischemia period. It is reasonable to assume that the oxygen free radical plays vital role in the development of some glaucoma. (supported by NSFC 3870621).

624.23

Studies designed to identify metabolic regulators of retinal blood flow (RBF) in the neonate eye are scant. We are investigating the hypothesis that adenose regulates neonate RBF. The present study was undertaken to determine if retinal vessels of the newborn pig eye respond to an intravitreal injection of 2-cloro-adenosine (2-CADO), an adenose agonist. Immuno- anesthetized, ventilated, pancuronium-paralyzed piglets (<5 days old) were used (n=5). The iris was dilated with 1% tropicamide, and the cornea and lens were removed and replaced with a glass cover slip to permit visualisation of the fundus via oivemicroscopy and xenon epi-illumination. Under 310x magnification, the visualization of the fundus via videomicroscopy and with 1% tropicamide, and the cornea and lens were re­

624.19
SITES OF BRAIN INVOLVEMENT IN A MURINE MODEL OF HERPETIC RETINITIS. J.C. Hedeen, J.S. Pepe*, and J.A. Whitsel, Dept. of Pediatrics and Ophthalmol., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205 and the Department of Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110.

When herpes simplex virus type 1, KOS strain, is inoculated into the anterior chamber of one eye in BALB/c mice, a virus infection develops in the anterior segment, but the retina remains almost normal. In contrast, the opposite uninjected eye develops a delayed necrotizing viral retinitis. To investigate possible paths of interocular transfer, we examined the localization of virus in the brain by immunocytochemistry at 1-4 days postinoculation. At day three, antigen-positive neurons were observed in the ipsilateral Edinger-Westphal nucleus and vicinity and the ipsilateral oculomotor nerve root exit zone. At day four, neurons in the contralateral medial pretectal area, the ipsilateral suprachiasmatic nucleus, and the ipsilateral intergeniculate leaflet of the lateral geniculate body became positive.

624.20
PATTERN ELECTRORETINOGRAM & VISUAL EVOKE POTENTIAL (VEP) IN RETINITIS PIGMENTOSA (RP). L. Mishra and S. Baner*, University of Texas Health Sciences Center & Houston Eye Associates, Houston, TX 77030.

RP is a hereditary disease of photoreceptors, progressive in nature and primarily affects the peripheral retina. Since pattern electroretinogram (PERG) is believed to reflect the activity of the ganglion cells and is mostly evoked from the foveal retina like VEP, we analyzed PERG and VEP in patients with RP who retained only the central vision.

Ten patients (18 eyes) with RP were evaluated using VEP, PERG, PERG (Flash-ERG) and Goldmann visual fields. For VEP and PERG, the visual stimuli were 0.5° black and white checks with 98% contrast reversing at 2 and 8 Hz. The central retina (6° x 19°) was stimulated. PERG was performed using a Ganelf stimulator. At day eyes 5-10° of the central retina was spared on the visual field. In these eyes the photopic response was abnormal and PERG was extinguished. In 4 eyes, 30° of the central retina was spared. The photopic response was normal and PERG was present in these eyes. The scotopic ERG was abnormal in all the eyes. On the other hand, VEP was abnormal in only 5 eyes. VEP gave normal results in 13 eyes, although 9 of the same eyes tested abnormal using the PERG method. From these findings, it appears that: 1) normal PERG response is dependent not only on the foveal but also the parafoveal retina, 2) PERG is more sensitive than VEP in detecting wide-spread disease of the retina. (Partially supported by NIH EY 07024).
625.1 COMPUTER SYSTEM FOR MAPPING PHOSPHENES PRODUCED BY INTRACORTICAL MICROSTIMULATION OF VISUAL CORTEX

S. J. Givre, C. E. Schroeder, R. A. Bazarbache, W. Haertel, and J. C. Arezzo.

The objective of this study was to characterize the superposition of human neuromagnetic responses and the resolution of sources when responses evoked by single visual stimuli are compared with responses evoked by the simultaneous presentation of paired stimuli.

Two-dimensional difference of gaussians (DOG) stimuli were presented to three different field positions located 2.5° to the right of the vertical meridian and either 2.5° or 12° below the horizontal meridian. The size of the stimuli was adjusted according to the cortical magnification factor and stimuli were presented either separately or in simultaneous pairs. Neuromagnetic measurements (56-112 sensor locations) were recorded from four right-handed human subjects. Sources were localized by fitting instantaneous neuromagnetic field maps with a multiple-dipole, spherical model. The reduced chi-square statistic was used to determine model order and best-fitting dipole parameter values.

A preliminary analysis of the data suggests that initial responses (<160 ms) reflect superficial cortical presentations. For deeper presentations, two dominant sources were identified from the major waveform deflection at 150 ms: (1) a source in the left extrastriate cortex that was invariant across stimulus position, and (2) a source in left inferior occipito-temporal cortex that was invariant across stimulus position. For simultaneous presentations, a source in left occipito-temporal cortex was observed that was close spatial proximity.

A DEC PDP 11/23 computer has been programmed to provide control of optically isolated stimulators that are connected to an array of intracortical electrodes. Once thresholds stimulators are established for the electrodes then the position in visual space must be established to provide meaningful information to the subject. The fact that phosphens move with eye movement complicates most methods of mapping the absolute position of phosphens. Thus a pair-wise mapping procedure was implemented. Stimulation is applied first to a reference electrode and then to the electrode to be mapped. The subject inputs the direction of the vector from the reference electrode to the map electrode by positioning a joystick in one of 16 possible directions. A binary search, regulated by the directional input of the subject, through the phosphens already mapped determines the neighbors of the phosphenes to be mapped. The final position of the phosphenes is determined through the use of a weighted average of two directional pairs. As each phosphenes is mapped it is displayed on a video monitor so that the investigator can observe the developing map.

625.2 QUANTITATIVE STUDIES IN MIGRAINE PHOSPHENES

O. J. Grüsser, U. Grüsser-ConRad.

Societies for Neuroscience, Abstracts, Volume 17, 1991

625.3 TEMPORAL ORGANIZATION OF THE VISUAL SYSTEM IN THE AWAKE MACAQUE.

C. E. Schroeder and S. J. Givre.

We investigated the organization of the primate visual system by comparing the timing of responses to standardized luminance and pattern (in fixating animals) stimuli across LGN, V1, V2, V3, and inferotemporal (IT) cortex. Timing was measured using laminar profiles of current source density and concomitant multiunit activity, recorded with multicontact electrodes in 9 awake, macaques. Luminance response latencies (range/mean) of animals sampled were:

- LGN, 14.24ms/17.5ms (n=5)
- V1, 20.5-30.5/27.6ms (n=5)
- V2, 23.73ms/26ms (n=3)
- V3a, 53.6ms/42-65ms (n=5)
- IT, 42-60ms/52.3ms (n=3)

Latencies to pattern reversal are 3-16 ms longer than collocated luminance evoked latencies. In cortical areas, responses in superficial laminae lag the initial response by 4-10ms. The direct, luminance response has a minimum duration of about 35 ms in LGN, and 40 ms in V1. We conclude that:

1. The latency difference between V1 and V2 and between V1 and IT is consistent with sequential organization of processing; and
2. The small latency differences between areas V1, V2, and V3a are consistent with the use of a partially sequential/hierarchical organization of processing in this portion of the system.

Despite the latency offset between early responses in LGN and V1, and later responses in IT, due to the long duration of activity at each point, processing throughout the system is broadly concurrent. Thus, feedback from extrastriate cortex influences visual processing in V1 and even in LGN, during the time frame of the response to a single stimulus. (Supported by MH37073 and Training Grant T32GM7288 from NIGMS.)

625.4 TIMING AND LAMINAR DISTRIBUTION OF ACTIVITY EVOKED BY WHITE LIGHT VERSUS COLOR IN V1 AND V4 OF THE AWAKE MACAQUE.

S. J. Givre, C. E. Schroeder, R. A. Bazarbache, W. Haertel.

Intracortical profiles of visual evoked potentials (VEP), concomitant multiunit activity (MUA) and current source density (CSD) were obtained from V1 (N=1) and V4 (N=2) of awake monkeys. 16-channel electrodes, capable of spanning the cortical laminae, were used for recording. Stimuli were full-field, stroboscopic flashes of white, red, blue and green light. In V1, white flash stimulation predominately activates lamina 4a, as indexed by current sinks and increased MUA in this layer. Successive decrements in flash intensity reduce the amplitude of the VEP and associated current sinks, and increase the onset latency of the response. Colored stimuli increase the amplitude of the VEP and the onset latency of the current sink in lamina 4cβ, but not 4cα, and in addition, produce a pattern of large current sinks of sequential onset in upper lamina 4 and lamina 3. Red produces the largest effect, approximately doubling the amplitude of the initial VEP component (N40-S50).

In V4, the initial response to white light is a brief, small current sink. As in V1, decreasing the intensity of the flash reduces the amplitude and increases the onset latency of the response. Colored stimuli prolong the initial sink without increasing its onset latency. In addition, they can prolong MUA or produce MUA bursts which were not reversible during white light stimulation. These data illustrate a surprising degree of differential processing of color versus white light full-field flashes both within the laminae of V1 and between V1 and V4. (Supported by MH37073 and T32GM7288 from NIGMS.)

625.5 SPATIAL DISCRIMINATION OF MULTIPLE NEUROMAGNETIC SOURCES EVOKED BY SEPARATE AND SIMULTANEOUS PRESENTATION OF VISUAL STIMULI.


The objective of this study was to characterize the superposition of human neuromagnetic responses and the resolution of sources when responses evoked by single visual stimuli are compared with responses evoked by the simultaneous presentation of paired stimuli.

Two-dimensional difference of gaussians (DOG) stimuli were presented to three different field positions located 2.5° to the right of the vertical meridian and either 2.5° or 12° below the horizontal meridian. The size of the stimuli was adjusted according to the cortical magnification factor and stimuli were presented either separately or in simultaneous pairs. Neuromagnetic measurements (56-112 sensor locations) were recorded from four right-handed human subjects. Sources were localized by fitting instantaneous neuromagnetic field maps with a multiple-dipole, spherical model. The reduced chi-square statistic was used to determine model order and best-fitting dipole parameter values.

A preliminary analysis of the data suggests that initial responses (<160 ms) reflect superficial cortical presentations. For deeper presentations, two dominant sources were identified from the major waveform deflection at 150 ms: (1) a source in the left extrastriate cortex that was invariant across stimulus position; and (2) a source in left inferior occipito-temporal cortex that was invariant across stimulus position. For simultaneous presentations, a source in left occipito-temporal cortex was observed that was indistinguishable from that seen for separate presentations. In the left extrastriate region two sources were observed from the separate conditions with opposite signs and some of their parameters differed from those in the separate conditions presumably due to their close spatial proximity.

625.6 A 8–12 Hz ("ALPHA") RHYTHM IN CAT VISUAL CORTEX

M. Chatila, C. Millertet, J.J. Bouyer, A. Rougeul, P. Buser.

In behaving cats, exploration of the visual and adjacent areas reveals several sets of electrophysiological rhythms. One of those displays the following characteristics: 1) its frequency lies in the "alpha" 8–12 Hz band; 2) it is interrupted by electrical stimulation; 3) it is located in area 18, or at the limit between areas 17 and 18, in the projection zone of the central vertical meridian. No such rhythm could be found in the most posterior part of the visual cortex nor in the posterior parietal cortex. It optimally develops while the animal is sitting for a significant target search, roughly similar conditions were previously shown to favour another set of rhythms, those in the somatic cortex ("mu rhythms"); coherence computation has however indicated that the two rhythms develop independently. Concerning its putative thalamic command zone, alpha recording electrodes were localized in the medial part of the lateral geniculate body, very close to nucleus lateralis posterior and pulvinar. We thus confirm that an alpha-like rhythm exists in carnivora, but with a restricted cortical localization, and that it is definitely independent of the animal's mu activity whose command zone is situated in n. ventralis posterior of the thalamus. Supported by DRET (Contract 89/069) and Fondation pour la Recherche Médicale.
625.7 INFLUENCE OF PUPIL DIAMETER ON PATTERN-ELICITED VISUAL EVOKEO POTENTIALS IN PIGMENTED RATS. W.K. Hoyes, F. Beidler, M.G. Drube, U.S. EPA, RTP, NC 27711.

Visual evoked potentials (VEPs) are a useful methodology for studying the neurotoxic and neuroactive properties of chemical compounds, testing stimulus intensity, and examining CNS actions, which may alter pupil diameter and thus effect both the refraction and luminance of the stimulus image on the retina. It is important to examine the effects of light on the intact visual axis. Injections of ganglion stimulating agents at 5 spatial frequencies (0.05, 0.1, 0.2, 0.4 or 0.8 cpd), 40% contrast, modulated in an on/off fashion with 5 Hz temporal sinusoidal. VEP waveforms were averaged over 100 trials, spectral analyzed, and spectral amplitudes at 5 (F1) and 10 (F2) Hz were measured. Preliminary data analysis suggested that neostigmine reduced F2 amplitude, and that tropicamide had little effect. A trend across drugs suggested that F1 amplitude was directly related to pupil diameter at certain spatial frequencies. Further studies should increase the available sample sizes and examine the influences of contrast and luminance.

625.9 CORRELATIONS OF EEG AND VEP SCOPOLAMINE EFFECTS IN HUMANS. N.G. SANSIVIERI, Center for neuroactive drugs, Institute of Neurophysiopathology, University; Center for cerebral neurosciences, CNB, Genova, Italy.

Acute 0.25-0.75 mg scopolamine reduced the amplitude of flash-evoked cortical potentials (VEP) (P2-N2 wave) and quantitatively background activity (EEG) in healthy humans. These effects were parallel in the 0-120 min postdose time-on-going and both related to dose; VEP/EEG correlation was across doses, within-dose correlation was restricted to 0.75 mg administration. Pattern-VEP were unaffected. To remove the EEG effect on flash/pattern-VEP amplitude non-linear regression vs the EEG total power was computed and VEP residua from the regression function were re-tested. Flash-VEP/EEG residuals replicated the original data as to post-drug timing and relation to dose while pattern-VEP were unaffected by this data processing. The results suggest the independence of scopolamine induced flash-VEP changes from those on background EEG signal. The effects of stimulus physical characteristics are to be considered to generalize the results in human neuropharmacology. Quantitative EEG monitoring in VEP drug studies allows inference with respect to the CNS activation levels and assessment of CNS drug effects on EEG and VEP variables unrelated to each other is practicable whenever interferences are excluded.

625.8 BACKGROUND WHITE NOISE ALTERS FLASH EVOKEO POTENTIALS (VEPs) IN RATS. D.W. Herr1, 2, D. King*3, W.P. Watkins1, 2, W.K. Hoyes, 2, U.S. EPA, RTP, NC 27711 and M.EIT, RTP, NC 27706.

We are examining how test procedures alter FEPs, and have found changes with daily noise stress in stimulus intensity, acoustic background noise, and test session (Dyer, R.S.; Physiol. Behav., 45:355-363, 1989; Herr et al., Physiol. Behav., 49:355-360, 1991; Herr et al., Soc. Neurosci. Abst., 16:570-1990). We report the impact of pressure stimulus on VEPs. Long-Evans rats were implanted with epidural electrodes over the visual cortex and subicantaneous ECG electrodes over the dorsal chest areas. Motor activity levels were recorded from the rectified and integrated signal on an exposed headset lead. Sequential 50 trial averages and test days were within-subject factors. On each of 9 days, 350 trials (60 min of noise) were presented. White noise (80 dB(A)) was presented either over trials 201-350 on day 9, over trials 1-350 on day 9, or over trials 1-350 on day 9. The presence of noise increased peak P30 and N70 amplitudes. When presented over trials 1-350, peak P30 and N70 amplitudes decreased. Peak N70 amplitude decreased initially, then increased, when noise was presented to naive subjects on day 9. Noise presentation over trials 201-350 decreased peak P30 latency. Motor activity was briefly increased and heart rate was not altered by noise. The data provide further evidence that non-visual variables play a role in modulating FEPs. Most of these effects appear unrelated to noise-induced effects on motor activity. DWH was supported by a NRC Research Associateship.

626.1 INTRACEREBRAL LHRH RELIEVES MATING BEHAVIOR DEFICITS CAUSED BY VOMERONASAL LESIONS: LHRH ANALOGS MAY EXERT AN EXTRA-PITUITARY EFFECT ON MATING BEHAVIOR IN MALE HAMSTERS. G. Fernandez*, G. Howard* and M. Meredith. Program in Psychobiology & Neuroscience, Dept. Biol. Sci., Florida State Univ., Tallahassee, FL.

Vomeronasal organ (VNO) sensory input is followed by the release of LHRH, which controls mating behavior in rodents of both sexes. Previous results (Meredith et al, Chem Sens 15:619) suggest that exogenous intracerebral LHRH might "substitute" for VNO input in sexual behavior restoration by intracerebral LHRH. Behavioral restoration by intracerebral LHRH was confirmed in VNX animals. LHRH, saline or an LHRH antagonist (NAL-GLU or Ac-LHRH 5-10) were given. VNX males were tested 30 min after injection and injection time, mounts, thrusts and intromissions were recorded. VNX animals, compared to controls significantly increased mating behavior (P<0.05) after LHRH (500g). Subcutaneous (500g) or intranasal (10ug) injection had no significant effect. NAL-GLU, a potent antagonist of pituitary LH release and Ac-LHRH 5-10, a fragment of LHRH that has no known activity, failed to significantly facilitate mating behavior in both VNX and CON animals (P>0.02) suggesting that analogs effective in facilitating mating behavior may act via receptors other than the pituitary type. Supported by Grants from NSF, BNS-8615159 & NIH, DC00906.


Response suppression and facilitation have been reported in orthodromic optical responses using paired stimuli. The presence of suppression or facilitation depends in the stimulus intensity as well as in the condition/test (c/t) intervals. In the present study, the spatio/temporal properties of the signals were studied by video imaging voltage-sensitive dye images, after orthodromic electrical stimulation of the olfactory nerve or of peripheral nerve fascicles. Low intensity stimulation which submaximally activates the olfactory bulb was necessary for the response facilitation. In addition, low temperature could enhance the size, duration and spatial distribution of these facilitated test responses. Depending on the intensity and c/t interval, different sectors in the same layer were selectively facilitated. Sectors which exhibited latency facilitation responses were facilitated at short c/t intervals and sectors which exhibited longer latencies at longer c/t intervals. Stimulation of particular regions of the mucosa evoked facilitated responses in different sectors of the olfactory bulb, depending on the c/t interval. The selective enhancement of activity in a particular sector reduced the activity in other adjacent locations. These data suggest a widespread, non-homogeneous, pattern of activation of the olfactory bulb, depending on the c/t interval. The spatial distribution of activity in the bulb is influenced by spatial as well as temporal patterns of activity in the olfactory nerve. Supported by NIH, Pew Freedom Trust, and Dept. of Neurosurgery.
626.3 ROLE OF STIMULATION FREQUENCY IN THE INDUCTION OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX. M. P. Gildergaard. S. Stripling, Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Repeated high-frequency stimulation of the granule cell layer of the olfactory bulb (OB) induces long-term potentiation (LTP) in the piriform cortex (PC), which is selective to late components of the evoked potential (Stripling, Patneau, and Gramlich, Brain Res. 542: 107-122, 1991). In previous research we have typically used a single brief pulse (BP) or a single train (BP) by stimulating the OB with trains of 10 pulses delivered at a frequency of 100 Hz. In the present experiment we examined the ability of 10-pulse trains varying in frequency from 5 to 400 Hz to induce selective LTP in the Medial Long-Dana region of the OB. Single and complex trains of 10 pulses delivered at a frequency of 100 Hz. In the present experiment we examined the ability of 10-pulse trains varying in frequency from 5 to 400 Hz to induce selective LTP in the Medial Long-Dana region of the OB. Single and complex trains of 10 pulses delivered at a frequency of 100 Hz.

626.4 ALTERED RESPONSE TO PAIRED-PULSE STIMULATION FOLLOWING INDUCTION OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX. S. Stripling, D. K. Patneau, and M. P. Gildergaard. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Stimulation of the olfactory bulb (OB) or lateral olfactory tract (LOT) evokes a potential in the piriform cortex (PC) whose initial component (A1) represents monosynaptic activation of pyramidal cells by the LOT. In a naive animal, paired-pulse stimulation produces potentiation of A1 due to CA1+ accumulation in synaptic vesicles. Repetition frequency stimulation of the granule cell layer of the OB induces a long-term potentiation (LTP) selective to late components of the evoked potential (Stripling, Patneau, and Gramlich, Brain Res. 542: 107-122, 1991). Although selective LTP leaves the expression of A1 unaltered following single-pulse stimulation, paired-pulse stimulation causes a depression rather than a potentiation of A1. (Stripling, Patneau, and Gramlich, Brain Res. 441: 281-294, 1988). This effect was investigated in male Long-Evans rats with chronically implanted electrodes by testing with paired-pulse stimulation before and after the induction of selective LTP. High frequency stimulation of the OB produced selective LTP and paired-pulse depression of A1. While high-frequency stimulation of the LOT produced neither effect. Paired-pulse stimulation that caused depression of A1 also depressed the pre-synaptic volley in the LOT. Even non-potentiated animals showed paired-pulse depression rather than potentiation of A1 if very short inter-pulse intervals were used. Taken together, these results suggest that paired-pulse depression of A1 following selective LTP is due to an enhancement of evoked inhibitory responses in the OB, resulting in activation of fewer mitral cells by the second pulse. This interpretation is consistent with previous evidence that selective LTP produces an enhancement of functional inhibition within the OB and PC.


To investigate the involvement of the forebrain on the neural code for gustation, the effects of reversible lesions of the gustatory neocortex (GN) on taste responses in the nucleus of the solitary tract (NTS) were studied in rats. Initial, taste-responsive NTS neurons were classified as "relay" or "non-relay" units based on the evoked response to electrical stimulation of the taste-responsive portion of the parahippocampal gyrus. Taste-responsive responses to taste stimuli lasted about the same amount of time as the duration of the stimulus, which varied from 5 to 400 Hz. Animals receiving stimulation frequencies of 5 or 10 Hz failed to show temporal overlap of the potentials evoked by each stimulus pulse. Previous research has demonstrated that affective stimulation evokes an NMDA-mediated potentiation in hippocampal slices only at stimulation frequencies comparable to those that produced LTP in the present study (Collingridge, Herron, and Lester, J. Physiol. 399: 301-312, 1988). These observations are consistent with the involvement of NMDA receptors in selective LTP in the PC. Supported by NSF grant BNS 85-19700 and the Marie Wilcox Howells Fund.

626.6 TASTE NEURONS OF THE HAMSTER SOLITARY NUCLEUS: VARIATION IN IMPULSE RATE AND AMPLITUDE. T.P. Hettinger*, L.D. Savory* and M.E. Frank. Dept. of Biostructure & Function, Univ. of CT Health Center, Farmington, CT 06030.

In extracellular recordings of taste-responsive neurons of the solitary nucleus (SN) of the golden hamster (Mesocricetus auratus), neurons differ in their stimulus profiles, latencies and interspike interval patterns. We have found that some SN neurons also show variation in impulse rate and amplitude. We recorded single-unit activity of SN neurons responding to anterior tongue stimulation, using micropipettes with 1-2 am tip filled with 4% HRP and 0.5 M KC1 in tris buffer, pH 7.6. Recording sites identified by HRP injections were located within the central and lateral subdivisions of the rostral SN, regions containing elongate, stellate and tufted cells and the preponderance of chorda tympani (CT) afferent endings. Neurons responding maximally to 0.1 M sucrose typically displayed bursting patterns of impulses, with as many as 20 impulses per burst. Bursting responses to sucrose have been found also in CT neurons rates (greater than 100 Hz), sucrose-responsive SN neurons often showed serial attenuation of amplitude within each burst, in some cases near to the point of extinction. Impulse attenuation has not been observed in CT fiber recordings. SN neurons responding maximally to 0.03 M NaCl or 0.1 M KC1 did not generally show bursting or spike attenuation. The unique pattern of impulse rate and amplitude variation seen in sucrose-sensitive SN taste neurons probably reflects the incoming afferent signals and the integrative properties of the impulse generating sites, and may be important for the coding of sweet taste. NIH grant DC00853.


Taste-responsive neurons in the nucleus of the solitary tract (SN) of the golden hamster (Mesocricetus auratus) generate two distinct interspike interval (ISI) patterns: single unique patterns and complex multidimensional ISI patterns. We attempted to simulate simple and complex ISI patterns with computer-generated spike trains, using a random number generating function to place spikes in 1-ms bins. First approximations of simple ISI patterns were obtained with examples at one (eg. 2.5 ms) response rate, but complex NTS ISI patterns were obtained with examples of at least two independent rates (eg. 2.5 ms, 50 ms). More exact simulation of neural responses may require consideration of refractory periods and changes in the expression of A1 unaltered following single-pulse stimulation, paired-pulse stimulation causes a depression rather than a potentiation of A1. Although selective LTP leaves the expression of A1 unaltered following single-pulse stimulation, paired-pulse stimulation causes a depression rather than a potentiation of A1.

626.8 EFFECTS OF DEPHASING OLFACTORIAL INPUT FROM RESPIRATORY CYCLE ON FIRING PROPERTIES OF OLFACTORIAL BULB NEURONS. E. Sobel and D.W. Tank. Biophysics Research Dep., AT&T Bell Laboratories, Murray Hill, NJ 07974.

Single unit studies have demonstrated that mirtal cells in the mammalian olfactory bulb can change the temporal structure of action potential firing in response to olfactory input without changing mean firing rate. Under natural breathing, the action potentials "bunch," becoming more frequent during the inspiratory phase and less frequent during the expiratory phase. There is uncertainty over the relative contributions to this phenomenon from, 1) the phasic nature of the olfactory stimulation and, 2) central reflex control, synchronized to the respiratory rhythm. To examine this issue, we have developed an experimental preparation that provides cyclical olfactory stimulation synchronized in frequency to the natural respiratory rhythm, but shifted in phase. We have examined the respiratory patterning of olfactory bulb unit activity while altering the relative phase of odor stimulation and the natural breathing cycle in anesthetized rats.

Preliminary results suggest that the relative phase of on-going tracheal breathing does not affect the single unit activity evoked by phasic stimulation of the olfactory epithelium. Consistency of action potential "bunching" appears to be produced by the phasic nature of the stimulation and the inherent dynamics of the circuitry of the olfactory bulb.
CHANGES IN INFORMATION FLOW IN DISSOCIATED MOUSE Olfactory bulb Cell Cultures. J. West and S.P. Frasca Jr. Department of Biological Sciences and Center for Neuroscience, University of North Texas, Denton, TX 76203.

Bursting dominates the spontaneous electrophysiological activity of neural networks formed from cultured dissociated mouse olfactory bulb cells. Measurements of various burst characteristics (e.g. duration, interval, period) are assumed to be a finite first order Markov chain (considering the relation of a burst to the following burst). A transition matrix is collected and the average stored information (ASI) is calculated using methods outlined by Shaw (The Dripping Faucet as a Model Chaotic System, Sana Cruz, CA: Aerial Press, 1989). ASI is a map from a data set to a real number representing the complexity of the system activity. Where ASI has power spectral and phase space reconstructions, this measure helps to present a more complete quantification of the states occupied by the network. Spontaneous bursting activity is typically ergodic and thus can be the behavior of at least 5-10 measurements into the future. Some aspects of bursting activity can be viewed as the function of a dynamical system. GASergic circuits may function as a control parameter, which can be modulated by bicuculline. As the concentration of bicuculline is increased, the ASI decreases, implying that the bursting activity is storing less and less information. This stored information is propagated less than three measurements into the future at high concentrations of bicuculline. These bicuculline effects are reversible. ASI compares favorably to the Lempel-Ziv characteristic exponent in the case of the logistic equation. Generalization to higher dimensions (multiple burst characteristics) and consideration of higher order Markov processes are planned. Potentially ASI may be used to identify functional subpopulations of cells, which may correspond to the various cell types found in the bulb.

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RAT PUPS CAN ACQUIRE NEUROBEHAVIORAL RESPONSES TO MORE THAN ONE ODOR. O. Cami and M. Leon. Department of Psychology, University of California, Irvine, CA 92717.

Exposure of rat pups to an odor during postnatal days (PND) 1-18 with concurrent tactile stimulation results in an olfactory preference. This odor preference appears to correspond with physiological, anatomical and neurochemical changes in the olfactory bulb. Since previous studies of this phenomenon used only one odor, we considered the possibility that the deprived odor relative to either air or novel odor. However, pups trained with both odors developed preferences to both odors relative to air and showed an equivalent contrast effect, i.e. they licked significantly less 0.1M sucrose than did the unshifted controls. In addition, following reward downshift, both groups significantly less 0.1M sucrose than did the unshifted controls.

This work was supported by grant HD 24236 to M.L.


In analogy with color vision and taste, it is a plausible hypothesis that the sensory quality of an odor is composed of a number of "primary odors." In the olfactory system the "primary odors" might be defined by the response properties of each of an unknown number of different classes of receptor neurons. If the response of each of the receptor classes to a particular odor can be described by a single numerical parameter, then the list of these numbers identifies the odor. It is therefore of interest to explore the limits and information processing requirements inherent in discrimination among odors specified in this way. We have approached this question by analysis of a simple model system. In this model an "odor" is simulated by a sequence of randomly generated numbers representing the responses of the receptor classes to the odor. A large number of these "odors" is then generated and stored as a table. The behavioral task of odor recognition is simulated by determining whether a matching procedure (linear correlation) can select the correct match between an arbitrarily selected test odor and the corresponding entry in the table. Numerical simulation and analysis demonstrate that, when the number of stored odors is plausibly large, reliable detection of a match requires many classes of receptor neurons. Supported by NINDC DC03474 and DAMD 86-C-605.

MODULATION OF OLFACTORY HABITUATION IN INFANT RATS BY A DEPRIVATION SENSITIVE NORDRAGREMA/CISEM/CM. Anderson, C.D. Kila, G.L. Miller and W.G. Hall. Dept. of Psychobiology, University of California, Irvine. CA 92717.

Deprivation of nutrients and maternal care affects activity and reactivity of infant rats (pups) and may decrease habituation to novel odors. Related to this deprivation effect is the finding of increases in noradrenaline (NA) turnover in the olfactory bulbs (OB) of adult rats which is implicated in decreasing habituation to meaningful odors (Dulzen & Ramirez, 1989). To quantify deprivation-mediated influences on odor habituation, we compared the ability of 12-day old rat pups to habituate to 15 presentations of a novel odor after 8-hr nutrient deprivation (POSTER), 8-hr deprivation of nutrients and maternal care (TOTAL), or no deprivation (CONTROL). In addition, NA turnover was estimated by assaying OB's of pups from the same conditions for [MHPG], the primary metabolite in rodent brain of NA. TOTAL pups exhibited more orienting in the first 3 blocks (3 trials/block) than the CONTROL pups and more orienting than CONTROL pups in the 2nd and 3rd blocks. Overall, TOTAL pups oriented more than POSTER pups in 4 out of 5 blocks and more than CONTROL pups in 3 out of 5 blocks. Matching the deprivation-induced changes seen in olfactory orientation changes were recorded in [MHPG] in the OB of individual pups from the same conditions; i.e., [MHPG] was only increased in the OBs of TOTAL pups, POSTER pups were not different from CONTROL. In summary, the TOTAL pups were slower to habituate to a novel odor than POSTER or CONTROL pups. MHPG is increased in TOTAL pups, but not in the other groups, suggesting that increased NA turnover may play a role in modulation of odor habituation at the level of the OB. Supported by NICHHD (HD17458).


During PND 1-18, all animals were exposed for 10 min/day to an odor with continuous perineal stimulation to peppermint odor only, orange odor only, or peppermint and orange odors on alternate days. On PND 19, the pups tested were for behavioral preference between either: (1) orange and air, (2) peppermint and air, or (3) peppermint and orange. Neural activity in response to either odor was examined in other pups by assessing 14C-2-deoxyglucose (2-DG) uptake in the olfactory bulb. Pups trained in an olfactory preference to that odor relative to either air or novel odor. However, pups trained with both odors developed preferences to both odors relative to air and showed an equivalent contrast effect. The preferred odors also elicited changes in the uptake of 2-DG in the olfactory bulb. This data suggest that early experience with more than one odor can successfully induce behavioral preferences and neural changes in the olfactory bulb.

This work was supported by grant HD 24236 to M.I.L.


Deprivation of nutrients and maternal care effects activity and reactivity of infant rats (pups) and may decrease habituation to novel odors. Related to this deprivation effect is the finding of increases in noradrenaline (NA) turnover in the olfactory bulbs (OB) of adult rats which is implicated in decreasing habituation to meaningful odors (Dulzen & Ramirez, 1989). To quantify deprivation-mediated influences on odor habituation, we compared the ability of 12-day old rat pups to habituate to 15 presentations of a novel odor after 8-hr nutrient deprivation (POSTER), 8-hr deprivation of nutrients and maternal care (TOTAL), or no deprivation (CONTROL). In addition, NA turnover was estimated by assaying OB's of pups from the same conditions for [MHPG], the primary metabolite in rodent brain of NA. TOTAL pups exhibited more orienting in the first 3 blocks (3 trials/block) than the CONTROL pups and more orienting than CONTROL pups in the 2nd and 3rd blocks. Overall, TOTAL pups oriented more than POSTER pups in 4 out of 5 blocks and more than CONTROL pups in 3 out of 5 blocks. Matching the deprivation-induced changes seen in olfactory orientation changes were recorded in [MHPG] in the OB of individual pups from the same conditions; i.e., [MHPG] was only increased in the OBs of TOTAL pups, POSTER pups were not different from CONTROL. In summary, the TOTAL pups were slower to habituate to a novel odor than POSTER or CONTROL pups. MHPG is increased in TOTAL pups, but not in the other groups, suggesting that increased NA turnover may play a role in modulation of odor habituation at the level of the OB. Supported by NICHHD (HD17458).
627.1

OPTICAL IMAGING OF CEREBELLAR AFFERENTS IN THE ISOLATED TURTLE CEREBELLUM. S.A. ELIAS, T.J. ERBNER, C. NICHOLSON, A. NEUROSCIENCE PROGRAM, UNIV. MINNESOTA, MINNEAPOLIS, MN 55455 AND DEPT. PHYSIOLOGY & BIOPHYSICS, NYU MEDICAL CTR., NEW YORK, NY 10016.

The flat, highly convoluted cerebellum of the turtle is superior to its highly convoluted mammalian counterpart for mapping afferent pathway distribution. Using optical recordings with a voltage-sensitive dye we observed the spatial patterns evoked by peduncular stimulation. Turtles were quickly decapitated and the entire cerebellum and peduncles removed. The tissue was stained with RH 795 for 30 minutes then placed, ventral side up, in a recording chamber below an epifluorescence microscope and imaged with a Photometrics CCD (14 bit A/D, 576X384 pixels). Stimulation of a peduncle with a tungsten microelectrode evoked a depolarizing optical signal that formed a narrow bundle, < 400 μm in width, which extended sagitally 4-5 mm from the electrode. Simultaneously recorded electrical field potentials were consistent with the image. The optical signal was recorded at various depths by changing the focus of the microscope. Signals were small (~0.02% change in fluorescence at 2.5X, 1.4 μm depth) but reproducible and consistent. Supported by NIH Grants NS-27210 (TJE) and NS-13742 (CN).

627.2

DEBLURRING OF SERIALLY SECTIONED IMAGES OF THE OPTICALLY RECORDED PARALLEL FIBER BEAM IN THE RAT CEREBELLUM. C. R. YAFFE, S.A. ELIAS, T.J. ERBNER, Dept. of Neurosurgery and Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

Serially sectioned images of neuronal activity detected with voltage-sensitive dyes contain significant amount of out-of-focus information. We have used a deblurring algorithm to improve the spatial patterns of neuronal activity in the X-Y plane. Rats were anesthetized with ketamine/xylazine, the cerebellar cortex exposed, placed beneath an epifluorescent microscope (3.5 X 0.200 n. a. d.) and stained with the dye RH 795. A parallel fiber beam was evoked with a tungsten micro-electrode. A series of images were taken with a Photometrics CCD camera, at 25μm increments of focus from the cortical surface to 350μm. Prior to deblurring, the depolarizing optical signal for the beam was observed throughout the entire depth series. However, depth profiles of evoked field potentials confined the Purkinje cell EPSP to a narrower range of depths, 90-150μm in extent. Application of the deblurring algorithm to this series of images restricted the appearance of the optical signal to the levels where the EPSP was recorded. Outside this range, the optical beam signals were absent. The net effect of the deblurring is to reduce the effective depth of field of the objective lens and reduce artifacts due to light scatter within the tissue. This method of restoration is a necessary prerequisite for three-dimensional analysis and rendering. Supported by NIH Grant #RO1-NS-27210.

627.3

DEVELOPMENT, PHARMACOLOGY, DISTRIBUTION, AND CELLULAR LOCALIZATION OF GABA, BINDING IN RAT CEREBELLUM. R. E. BURGESS, J.F. LORDEN, AND B. DAVIS. Dept. of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104.

Receptor autoradiography using [3H] GABA under selective conditions was used to characterize the development, pharmacology, distribution, and cellular localization of GABA binding sites in the rat cerebellum. Binding is greater in the molecular layer (ML) than in the granular cell layer at all ages and reached adult levels by postnatal day (PND) 14. A pattern of parcellar and dendritic localization was observed in the ML of lobules I-V in adult rats. Transient expression of high levels of GABA binding was observed in the deep cerebellar nuclei, peaking at PND 7 and decreasing to adult levels at PND 18. Drugs active at the GABA binding site displaced [3H]GABA with the following order of potency: 3-APA > S-ALA = saclofen = 2-hydroxyaclofen > phaclofen. GTP-y-S and 5'-dATP inhibited [3H]GABA binding in a dose dependent manner. The cellular localization of GABA binding was investigated using lesion techniques. Neither MAM nor 3-AP lesions resulted in a decrease in [3H]GABA binding in the ML. These results suggest that the majority of GABA binding sites in the ML are located on Purkinje cell dendrites. Supported by NS-19613, NS-01300, MH-14279.

627.4

EXCITATORY AND INHIBITORY AMINO ACID BINDING SITES IN HUMAN DENTATE NUCLEUS. R.L. ALBIN, R.H. PRICE, S.Y. SAKURAI, J.B. PENNEY, AND A.B. YOUNG. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, Michigan 48104-1687.

We used receptor autoradiography to study the distribution of excitatory and inhibitory amino acid binding sites in human dentate nuclei. There was no binding to N-methyl-D-aspartate (NMDA) or GABA binding sites, and there was a low level of kainate binding sites. α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid, metabotropic quisqualate, benzodiazepine, and GABA Binding sites were present in moderate abundance. Our NMDA results are different than those found in rodents. CABA receptors are probably the primary mediators of inhibitory neurotransmission within the deep cerebellar nuclei while α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and metabotropic quisqualate receptors are probably the primary mediators of excitatory neurotransmission within the human deep cerebellar nuclei.

Supported by USPHS grants NS01300, NS15655, NS19613, and AG08671.

627.5


Both dn rats and rats with 3-acytylpiridine (3AP) lesions of the inferior olive displayed dystonia. Immunohistochemistry in the dentate nucleus (ICN) gluantamate decarboxylase (GAD) activity. GAD, the synthetic enzyme for GABA, is located primarily in Purkinje cell terminals. In 3AP rats, increased GAD activity is coincident with increased Purkinje cell activity, following destruction of the climbing fibers. The dn rats, however, show lower than normal Purkinje cell activity. Immunocytochemistry was used to determine whether dn and 3AP rats showed the same pattern of GAD activity in the ICN. The size and density of GAD + puncta were examined in three groups of 20-day-old rats: dn, normal saline-treated, and normal rats given 3AP (15 mg/kg) at 18 days of age. Separate groups of 3AP and saline controls were also examined at 14 days postinjection. An increase in the size and the number of GAD + puncta in comparison with normal rats was seen only in the 3AP group at 2 days postinjection when GAD levels, as measured by HPLC, were also increased in the 3AP group. An increase in size of GAD + puncta in size was no longer present at 14 days postinjection, when GAD activity is still elevated but Purkinje cell activity has declined. Puncata density was reduced in the dn rats only. Other studies show that GAD activity remains elevated in 3AP rats after Purkinje cell activity declines. The dn rats resemble 3AP rats following extended survival. In both cases, increased GAD activity may represent a neuroprotective response. The decrease in GAD activity may be related to a shift in the function of GAD that predominates in Purkinje cells at different times. (Supported by a grant from the Dysstonia Med. Res. Fdn. and grant BNS 90-15187 from NSF.)

627.6

RELATIVELY SMALL NUMBERS OF PONTOCEREBELLAR AXONS PROVIDE COLLATERALS TO THE CEREBELLAR NUCLEI. G.A. MIHALOFF, Dept. of Anatomy, University of Minnesota Medical Center, Minneapolis, MN 55455.

An issue that continues to be relevant to motor control studies is the question of whether information being transferred from sensorimotor cerebral cortex to the cerebellar cortex via the basilar pontine nuclei (BPN) is also distributed to other cerebellar areas. There is little data in the literature to support such a connection, yet schematic diagrams depicting cerebrocerebellar communication often include such circuitry. As an extension of ongoing research in our laboratory focused on the structure and function of the BPN, we have undertaken a study of this question using the orthograde axonal transport of the lectin Phosvitin. After a survival period ranging from 3 to 5 days, rats were sacrificed and sections of the cerebellum reacted for the presence of PHAL using a biotin-avidin immunocytochemical procedure. Our observations indicate that only a small number of pontocerebellar axons give rise to collaterals that terminate in the CN, and of those that do so, the majority arise from the NTAD and go to the BPN. Accumulations of labeled fibers and terminals are found in the ventral and lateral portions of the lateral cerebellar nucleus, the posterior interpositus nucleus, and portions of the medial cerebellar nucleus. These findings suggest that sensorimotor neurotransmission does not go to a relatively direct route to the CN but rather is transmitted to the CN after passage through the cerebellar cortex. Although other pontocerebellar nuclei such as the lateral reticular nucleus receive sensorimotor cortical afferents, the axons do not project directly to the CN but rather are transmitted to the CN after passage through the cerebellar cortex. Supported by USPHS grant NS12644.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
627.7 CORTICOCEULAR AND -VESTIBULAR PROJECTION ZONES CORRESPOND TO ZEBRIN-POSITIVE AND -NEGATIVE ZONES IN ANTERIOR VERMIS OF THE RAT CEREBELLM. C. J. Voogd, E. L. Elemen, J. G. R. Haaker (1), D. T. J. N. Oosterveer (1), R. J. W. Eimerman (1), U. van den Brink (1). (1) Dept. of Anatomy, Erasmus University, Rotterdam 3000 DR The Netherlands. Supported by the Netherlands Organization for Scientific Research (NWO). We have used a combination of electrophysiological and immunohistochemical techniques to investigate the extent to which olivocerebellar terminations to the anterior lobe of the rat cerebellum are characterized by the presence of zebrin-positive and zebrin-negative cells. Zebrin-positive cells were defined as those which showed a positive reaction to the lectin WGA-HRP when injected into the inferior olive and retrogradely labeled in the cerebellar cortex. Zebrin-negative cells were defined as those which showed a negative reaction to the lectin WGA-HRP when injected into the inferior olive and retrogradely labeled in the cerebellar cortex.

627.8 BRANCHING IN THE ROSTROCAUDAL AXIS WITHIN THE PROJECTION FROM THE INFERIOR OLIVE TO THE C2 zone of the CAT CEREBELLM. D.L. Tolbert, K. Yates-Silata, M.G. La Regina, L. Woods, T. Pittman, and D.L. Tolbert. Barrow Neurological Institute, Phoenix, AZ 85013. The inferior olive (JO) is a major source of efferent input to the cerebellum and plays a critical role in coordinating activity of the brainstem reticular formation that produces heavy anterograde label in C3-C4 propriospinal neurons. The largest percentage decrease in area occurred in the C3-C4 propriospinal neurons. The largest percentage decrease in area occurred in the C3-C4 propriospinal neurons. The largest percentage decrease in area occurred in the C3-C4 propriospinal neurons. The largest percentage decrease in area occurred in the C3-C4 propriospinal neurons.

627.9 THREE-DIMENSIONAL VISUALIZATION OF THE INTERNAL MEMBRANE SYSTEM OF AVIAN PURKINJE CELL DENDRITES. M. E. Martone, Y. Zhang, V. M. Simpliciano, B. D. Carragher, and M. H. Elemen, San Diego Microscopy and Imaging Resource, Department of Neurosciences, University of California, San Diego, La Jolla, CA 92030-0608. We have used high voltage electron microscopy (HVEM) to determine whether the differential distribution of these proteins is due to the presence of selectively stained membrane systems viewed in thick sections with HVEM. We have used a high voltage electron microscope (HVEM) to determine whether the differential distribution of these proteins is due to the presence of selectively stained membrane systems viewed in thick sections with HVEM. We have used a high voltage electron microscope (HVEM) to determine whether the differential distribution of these proteins is due to the presence of selectively stained membrane systems viewed in thick sections with HVEM. We have used a high voltage electron microscope (HVEM) to determine whether the differential distribution of these proteins is due to the presence of selectively stained membrane systems viewed in thick sections with HVEM.

627.11 SPATIAL ORGANIZATION OF SPINOCEREBELLAR INPUT TO THE UNFOLDED CEREBELLAR ANTERIOR LOBE. D.L. Tolbert, K. Yates-Silata, and B.R. Clark. (1) Department of Anatomy and Neurobiology and Surgery (Neurosurgery), St. Louis Univ. Sch. of Med. and Program in Physical Therapy, Washington Univ. Sch. of Med., St. Louis, MO Lower thoracic-upper lumbar (LTL) projections to the cerebellar anterior lobe were studied in rats using WGA-HRP tractography labeling techniques and image analysis software (Bioquant) that graphically unfolds the cortex while maintaining spatial relationships between identified spinocerebellar (SpC) mossy fiber terminals and the targeted cerebellar surface. Small injections restricted to approximately one LTL segment labeled SpC terminals in lobules I-VI, but did not label the whole LTL segment. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site.

627.12 PRELIMINARY CHARACTERIZATION OF A MODEL OF HEREDITARY PURKINJE CELL-INFERIOR OLIVARY DEGENERATION IN RATS. K. Yates-Silata, M.G. La Regina, J. Woods, T. Pimman, and D.L. Tolbert. Dept. Anatomy and Neurobiology, Comparative Medicine, and Surgery (Neurosurgery), St. Louis Univ. Sch. of Med., St. Louis, MO The cerebellum and brainstem of adolescent and young adult Sprague-Dawley rats were studied using WGA-HRP tractography labeling techniques and image analysis software (Bioquant) that graphically unfolds the cortex while maintaining spatial relationships between identified spinocerebellar (SpC) mossy fiber terminals and the targeted cerebellar surface. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site. The injection site was compared to the compartmentalization for zebrin and the injection site.
CALCITONIN GENE-RELATED PEPTIDE IN THE CAT'S CEREBELLUM. GEORGINA A. BISHOP. Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

In the present study, the distribution, origin and physiological effects of calcitonin gene-related peptide (CGRP) are examined in the cat's cerebellum. The PAP technique revealed that CGRP-immunoreactivity (IR) is present in profiles that have morphological characteristics of mossy fibers. These CGRP-IR mossy fibers have the lateral reticular nucleus, external cuneate nucleus, inferior vestibular nucleus and basilar pons. Physiologically, CGRP has a weak suppressive effect on spontaneous activity in most Purkinje cells. In addition, this peptide decreases the level of the cells' excitatory response to simultaneous application of glutamate or aspartate. Occasionally, CGRP increased neuronal excitability. Taken together, these data indicate that there is a chemically and physiologically distinct population of mossy fibers that arises from specific precerebellar nuclei. Functionally, CGRP likely modulates Purkinje cell activity in restricted regions of the cerebellar cortex by altering the responsiveness of these cells to the excitatory amino acids glutamate and aspartate. (Supported by NS18028).

DO TRANSIENT CLIMBING FIBERS PROVIDE A TEMPLATE FOR THE SEGMENTAL ORGANIZATION OF MOSSY FIBERS? JS. King and G.A. Bishop. Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The PAP technique was used to detect cholecystokinin (CCK) in the developing cerebellum of the opossum. In the adult, CCK immunoreactivity is present almost exclusively in mossy fibers (King & Bishop, 90, JCN 298:373). During development, CCK-positive fibers are present in the cerebellar peduncle by postnatal day (PD) 1, where they wait and then penetrate the cerebellar anlage on PD 7. The axons arising from these fibers remain in the medullary core of the cerebellum until PD 26. At that time, in the anterior lobe, some CCK-positive mossy fibers target the internal granule cell layer (IGL). At this stage of development, PK cells are in a layer 4-5 cells deep. Most of these immunoreactive elements are transient as they do not persist beyond PD 68. A population of internal olivary neurons also transiently expresses CCK during this time interval. Prior to the appearance of these transient elements, all granule cell migration bands of CCK-positive immunoreactivity are present. A population of cerebellar peduncle CCK-positive fibers do not penetrate the PK cell layer. Rather, they remain in the medullary core of the cerebellum until the internal granule cell layer (IGL) can be differentiated histologically (PD 33). Coincident with this developmental event, CCK axons grow into the IGL. Arsenio-Nunes et al. ('88, JCN 273:120) have suggested a primary role for the Purkinje cell in the establishment of CCK-immunoreactive mossy fibers and granule cells. Whereas CCK-positive mossy fibers were identified in the present study, mossy fibers or parallel fibers were identified in a double label technique revealed that CGRP-IR mossy fibers arise from neurons located in the external cuneate nucleus, inferior vestibular nucleus and basilar pons. Physiologically, CGRP has a weak suppressive effect on spontaneous activity in most Purkinje cells. In addition, this peptide decreases the level of the cells' excitatory response to simultaneous application of glutamate or aspartate. Occasionally, CGRP increased neuronal excitability. Taken together, these data indicate that there is a chemically and physiologically distinct population of mossy fibers that arises from specific precerebellar nuclei. Functionally, CGRP likely modulates Purkinje cell activity in restricted regions of the cerebellar cortex by altering the responsiveness of these cells to the excitatory amino acids glutamate and aspartate. (Supported by NS18028).


By avidin–biotin peroxidase immunohistochemistry, choline acetyltransferase (ChAT) and corticotropin–releasing factor (CRF) were examined in cat vestibular nuclei and related cell groups. Surgery was conducted on adult cats under general anesthesia with intraperitoneal choline acetyltransferase (ChAT) and corticotropin-releasing factor (CRF) CEREBELLAR MOSSY FIBER PROJECTIONS. In the vermal lobules I, II, IX and X, most of the ChAT and CRF neurons in the vestibular area were retrogradely labeled by the tracer. The results suggest that the vestibular area contains two independent populations of vestibulo-cerebellar projection neurons chemically coded by ChAT and CRF.

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626.3 ACUTE UNILATERAL SENSORIMOTOR CORTEX INJURY IN RATS BLOCS D-AMPHETAMINE INDUCED NEURONAL FUSION IN CEREBELLUM. J. B. Goldenstern and Y. MacMillan. V.A. & Duke Medical Centers, Durham, NC 27705.

Pharmacologic studies have implicated norepinephrine (NE) in amphetamine-facilitated motor recovery following sensorimotor cortex (SMC) injury in rats. We studied the acute effects of unilateral SMC ablation on the release of NE in the cerebellum in vivo by microdialysis. Dialyse samples were collected in 10 min fractions and NE assayed by HPLC-ECD. All studies were carried out in anesthetized rats.

Baseline levels of NE were undetectable in the cerebellar diasylate. The administration of a single dose of d-amphetamine (2.6 mg/kg base weight, i.p.) resulted in a significant increase in dialysate NE levels which peaked 30 min post dosing (15 pg/µl, not corrected for recovery). However, the administration of the same dose of d-amphetamine to naive rats 60 min. following a suckling-ablation lesion resulted in right SMC did not result in NE release into the cerebellar diasylate.

This data provides evidence for an acute remote effect of SMC injury on amphetamine-induced NE release in the cerebellum (diaschisis). Experiments are in progress to establish the duration of this effect.

Supported by the N.I.H. (NS 01162) and the V.A.

626.5 IMAGING OF REGIONAL VARIATIONS OF ELECTRICALLY EVOKED SODIUM INFUX IN CEREBELLAR PURKINJE CELLS. N. Lasser-Ross and W. N. Ross. Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

We used the fluorescent sodium indicator SFBI to monitor changes in [Na+] associated with intrasomatically and synaptically stimulated events. Neurons in sagital slices from the guinea pig cerebellum were injected with the impermanent dye BAPTA and a sodium electrode also used for stimulating and recording. Changes in fluorescence, excited at 380 nm, were recorded with a high speed CCD camera.

Intrasomatically evoked bursts of fast action potentials caused an increase in [Na+] limited to the soma and axon. The optical signal was proportional to the number of fast action potentials. The change in [Na+] (measured by AF/FP) was much larger, and the recovery time much faster (τ < 1 ms), in the axon than in the soma. The large surface to volume ratio of the axon is probably one factor determining these differences. There was no detectable change in [Na+] in the dendrites corresponding to either Na or Ca dependent action potentials. The lack of signal also shows that SFBI was not responding to changes in [Ca].

Clamping Fiber activation caused a small change in [Na+] in the lower part of the dendritic tree. The signals showed paired pulse depression consistent with whole cell recordings (Pseudemys scripta elegans) cerebellum. Fluorescence changes were imaged with a high speed CCD camera. Voltage dependent Ca influx in the soma was mostly correlated with fast, TTX-sensitive spikes. Ca transients in the spiny dendrites were correlated with calcium spikes and had much faster rise and fall time. In some cases the location of Ca influx varied from spike to spike implying localized and variable spiking in the dendrites. In these aspects turtle Purkinje cells resemble guinea-pig Purkinje cells. When an external electric field was applied across the cerebellum Ca spikes were evoked with Ca influx confined to the tips of the spiny dendrites demonstrating that the direction of field firing can take place in the spiny dendrites. Both PF and CF stimulation evoked voltage dependent Ca influx. The distribution and magnitude of synapticly evoked Ca influx was dramatically affected by a 4-AP sensitive A-like potassium conductance (Chan, Hougaard & Midtgaard, J. Physiol. 409, 143-156, 1989).

Supported by NS16295 and BNS-8819188.
628.7
ELECTROPHYSIOLOGICAL PROPERTIES OF IDENTIFIED GRANULE CELLS. C. Huang, C.-F. Hsieh, and R. Huang*. School of Basic Life Sciences, Univ. Missouri-Kansas City, Kansas City, MO 64110.

The cerebellar granule cells provide important sources of excitation for all cell types in the cerebellar cortex. Yet the electrophysiological properties of granule cells remained poorly known. Indeed, it was unclear whether the somata of mature granule cells possess the necessary combination of ion channels to generate conventional action potentials. We have recorded single granule cells intracellularly followed by horseradish peroxidase injection in cats under chloralose anesthesia (70mg/kg). The site of recording and injection was confirmed to be at the soma of the injected granule cell. The resting membrane potential was -43.5±6.7mV (n=31). The action potentials were 7.7±2.6mV extracellularly and 53.4±15.8mV intracellularly (n=22). Among the 31 granule cells, 26 responded to sound (60dB above threshold) with average latencies at 24.9±8.2ms while 17 responded to light with average latencies at 53.0±11.0ms. Five granule cells, however, did not respond to either sound or light. The mean inter-spike interval of granule cells was 152.7±68.8ms (n=21). (Supported by PHS grant AA07645).

629.1
A NEURAL NETWORK FOR LEARNING MUSCLE COORDINATION.
J.B. J. Smeets*, and J.J. Denier van der Gon*.

In the human arm, there are more muscle pairs than degrees of freedom. To make a specific movement, the nervous system has ample choice from combinations of muscle activations. We present a neural network model which learns, unsupervised, from afferent signals to coordinate muscles in a realistic way.

The model consists of a planar arm with two joints, controlled by four mono-articular and two bi-articular muscles. As a substitute for a homogeneously innervated motoneuron pool we used one motoneuron. A pooled afferent signals muscle stretch. An array of central neurons innervates the motoneurons. Movements are generated by the activation of abutting central neurons. All central neurons and all afferents are connected to all motoneurons. The weights of these connections are adapted during centrally induced movements of the arm, using only information present at the location of that neuron. Simulations start with poor coordination: each central neuron has only one non-zero connection with a motoneuron.

The resulting coordination of the muscles conforms to experimental data on muscle activation. Furthermore, the model predicts homonymous and heteronymous monosynaptic reflexes in a realistic way, for instance a heteronymous reflex in the mono-articular elbow flexor when shoulder-flexors are stretched.

629.3
EFFECTS OF DORSAL ROOT CUT ON FORCES EVOKED BY SPINAL STIMULATION IN SPINALIZED FROGS.
E.P. Loeb, S.F. Giszter, E. Bizzi, and F.A. Mussa-Ivaldi*.
Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Recent experiments in spinal frogs have shown that focal microstimulation of a single site in the lumbar grey, in conjunction with positioning the leg in different workspace locations, results in the generation of a force field with a single equilibrium point. The equilibrium point is that spatial location at which the leg would be at steady state were it free to move. The force fields are produced by spinal circuits and modulated by afferent feedback. The purpose of this work was to investigate the contribution of afferent feedback to the force fields. We measured the force fields before and after cutting the dorsal roots without moving the stimulating electrode. After cutting the dorsal roots we found that the current threshold required to induce a force field was temporarily elevated, and when induced the dynamics of the rise and fall of the force traces was altered. This suggests a role for afferent activity in the maintenance and shaping of the force fields. We will present a quantitative analysis of the effects of the dorsal root cuts on the shape of the force traces.

This work was supported by NIH grants NS09343 and AR26710, and ONR grant N00014-96-0872.

629.2
THE RELATIONSHIP BETWEEN LATERAL DOMINANCE AND TURNING PREFERENCE ON THE STEPPING TEST.
Fred H. Previc and Joanna Saucedo*.
Armstrong Laboratory, Brooks AFB, TX 78235-5001.

Previous research has shown a relationship between handedness and the direction of turning during Fukuda's stepping test. This study attempted to correlate directional preference on the stepping test with several measures of lateral dominance, including handedness. A total of 113 subjects from a local high school participated as subjects. The vast majority of subjects (89%) were right-handed, while a smaller percentage (11%) were left-handed and right-eyed. Subjects performed the stepping test with their vision occluded and their arms folded in front of them, and their angular rotation during a 1-min interval was measured. Although only a slight majority of subjects turned rightward, the direction and magnitude of turning correlated significantly with footedness and eyedness (but not handedness). For example, 66% of right-footed and right-eyed subjects turned rightward, whereas less than 40% of subjects with left and mixed preferences did so. The results indicate that axial turning preferences and various lateral dominance tendencies may at least partially share a common mechanism, possibly involving the vestibular system.

629.4
DISCRIMINATING AMONG RHYTHMICAL BEHAVIORS IN THE CHICK.
R.M. Johnson, S.M. Woolley, M.B. Smith and A. Bekoff. Dept. of E.P.O.
Biology, University of Colorado, Boulder, CO 80309-0334.

Our intent in this study was to quantify the degree of relatedness among different rhythmical hindlimb behaviors using discriminant function analysis. We chose to compare the kinematic profiles of five behaviors: walking, swimming, ataxoping, foot shaking and head scratching. The following results do not yet include head scratching.

Small dots of black ink placed on the skin overlaying the lateral surface of the right hindlimb were used to define the hindlimb and the hip, knee and ankle joints. Video recordings were made as chicks produced one of the five behaviors being examined. The hindlimb movements were digitized and we quantified several features of intra- and interjoint coordination patterns. Discriminant function analyses were performed on groups of non-complementary variables using a cross validation approach for behavioral classifications.

Our results show that functions based on the actual time (ms) of kinematic features discriminate unilateral foot shaking from the three bilateral behaviors. We conclude that this separation corresponds to a cycle duration constraint between unilateral (max ≤ 150 ms) and bilateral behaviors (min ≥ 150 ms). When variables were expressed as a proportion of their cycle durations, converting real time to relative time, functions discriminate walking, which involves weight support, from the other three behaviors which lack weight support. When walking is removed from these analyses relative latencies and not relative durations discriminate among the remaining three closely related behaviors. These results suggest that the behavioral implications of weight support are dramatic and that discrimination among the more closely related behaviors may reflect different interjoint coordination patterns in each behavior. By establishing the relatedness among diverse behaviors we provide the foundation for interpreting the differences in the motor patterns underlying these behaviors. Supported by NIH grant NS20310.
629.5


Fluctuations in the amplitude of the EMG envelope throughout a step cycle have been reported for locomotor tasks and is an apparent representation of the smoothed EMG activity. It reflects the frequency of discharge of motor units in a muscle (Hoffner et al., J. Neurophysiol. 57:330, 1987). Thus EMG waveforms can be interpreted to be indicative of the number of motor units that are being activated. The primary advantages of the EMG technique are that it is non-invasive and can be used in both human and animal experiments.

In the current study, the EMG activity of the biceps brachii and triceps brachii muscles was recorded during treadmill locomotion. The EMG activity was measured using surface electrodes placed over the muscle belly. The EMG signals were amplified, filtered and rectified before being recorded on a computer. The EMG amplitudes were analyzed and compared for different locomotor tasks.

Findings

1. The EMG amplitudes for the biceps brachii and triceps brachii muscles were significantly higher during fast locomotion compared to slow locomotion.

2. The EMG amplitudes for the biceps brachii and triceps brachii muscles were significantly higher during walking on a treadmill compared to walking on a level surface.

3. The EMG amplitudes for the biceps brachii and triceps brachii muscles were significantly higher during running on a treadmill compared to walking on a treadmill.

4. The EMG amplitudes for the biceps brachii and triceps brachii muscles were significantly higher during a stop and go protocol compared to a steady walking protocol.

5. The EMG amplitudes for the biceps brachii and triceps brachii muscles were significantly higher during a stop and go protocol compared to a steady walking protocol.

6. The EMG amplitudes for the biceps brachii and triceps brachii muscles were significantly higher during a stop and go protocol compared to a steady walking protocol.

Conclusions

The EMG waveforms are a reliable measure of the changes in EMG activity during locomotor tasks. The EMG waveforms can be used to study the effects of different locomotor tasks on the muscle activity of the biceps brachii and triceps brachii muscles. These results can be used to develop new treatments for patients with neurological disorders such as stroke and Parkinson's disease.

Supported by NIH Grant NS16333.

629.6


The hindlimbs of adult spinal cats (T12-T13) can be used to study the effects of spinal cord injury on locomotion (Lovest et al. J. Exp. Neurol. 92:421, 1986; Barbeau and Rossignol, Brain Res. 412:84, 1987). Although the general pattern of EMG bursts of selected extensors and flexors appears to be similar in spinal and intact cats, some effects of spinalization are evident. In the present study, the EMG patterns were studied in the same cat during quadrupedal and bipedal stepping and during bipedal stepping on a treadmill. The EMG recordings were obtained in the cats with the spine prespinalization and the corresponding spinal patterns were recorded in the same cat during quadrupedal and bipedal stepping. The EMG waveforms were analyzed using a computer program that identified the peaks and troughs of the EMG activity. The EMG waveforms were compared to the corresponding spinal patterns recorded in the same cat during quadrupedal and bipedal stepping.

Findings

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Supported by NIH Grant NS16333.

629.7


The pharmacological effects on the EMG activity of the hindlimb flexors and extensors were studied in adult spinal cats. The animals were trained to walk on a treadmill and the EMG activity of the flexors and extensors was recorded during locomotion. The EMG activity was analyzed using a computer program that identified the peaks and troughs of the EMG activity. The EMG waveforms were compared to the corresponding spinal patterns recorded in the same cat during quadrupedal and bipedal stepping. The EMG waveforms were analyzed using a computer program that identified the peaks and troughs of the EMG activity. The EMG waveforms were compared to the corresponding spinal patterns recorded in the same cat during quadrupedal and bipedal stepping.

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Conclusions

The EMG waveforms are a reliable measure of the changes in EMG activity during locomotor tasks. The EMG waveforms can be used to study the effects of different locomotor tasks on the muscle activity of the biceps brachii and triceps brachii muscles. These results can be used to develop new treatments for patients with neurological disorders such as stroke and Parkinson's disease.

Supported by NIH Grant NS16333.

629.8

PERCEPTUAL AND Nystagmographic RESPONSES TO BODY TURNING. V.R. Edgerton. Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1527.

Somatic sensory and motor inputs from active stepping in a circle elicit compensatory nystagmus with a gradual rise in slow phase velocity (SPV) in normal individuals. We simultaneously assessed in seated subjects how nystagmus and perceived self-motion (PSM) are influenced by pedalling or dragging movements of the feet.

Subjects (N=10) sat in a rotating chair in darkness and were turned for 30 s at 120 deg/s. For 45 s following the turn, 2.5 or 5 Nm extensor torque was provided by a motor, patients were able to perceive that they were turning; however, their body sway did not increase. The results of this study suggest that the SPV is the critical variable for normal perception of self-motion.

Supported by NASA Grant N9-295.
Previous speed-accuracy investigations have identified relationships between movement time (MT) and peak velocity (PV) and extrinsic variables (i.e. displacement, accuracy requirements, contextual demands). This investigation demonstrated that biomotoric and extrinsic variables also may affect MT and PV. Four subjects performed two types of pointing movements, reaches (shoulder and elbow moving in opposite directions) vs. whips, (shifting in same directions) under two conditions, fast vs. fast/accurate. These multijoint arm movements were compared across three amplitudes of displacement (10.16cm, 20.32cm, 40.64cm) in the horizontal plane. As expected, we found: (1) MT and PV were linearly related to displacement, and (2) accuracy demands increased MT and decreased PV. However, MT was briefer and PV was higher for whips under both fast and fast/accurate movements of comparable displacements. These MT/PV differences between whips and reaches are not accounted for by Fitts' Law (Fitts, P.M., J.E.P. 47; 381,1954) or subsequent modifications (Schmidt, R.A., et al., Psychol Rev 86:415, 1979; Meyer D.E. et al., Attention and Performance XIII, 173, 1990). Therefore, the speed as a movement parameter is not only on the accuracy and distance requirements of the task but also on biomechanical factors, such as, the summation of interactional forces.
629.17 ANIMAL MODEL OF OPTIC FLOW UTILIZATION IN THE CONTROL OF LOCOMOTION. H. Sun*, D. P. Carey, and M. A. Goodale. University of Toronto, Ontario, Canada.

In spite of numerous claims of flow-field processing in visual neurons of the CNS (Steinmetz, et al., 1987, J. Neurosci.; few investigators have manipulated optic flow to examine its effect on behavior. Although there have been some laboratory studies of the use of optic flow, none have demonstrated its use in a free-living, non-human animal. Our study involves normal adult cats and suggests that TP can be controlled by the spinal cord. Our study of Physical and Occupational Therapy, McGill Univ., Montreal, PQ, Canada H3G 1Y5.


Interest in the primate motor system was dominated during the early and middle parts of this century by such renowned scientists as Sherrington, Fulton, Hines, Tower, Woolsey, Mettler, Bucy, Binnay-Brown and Kuppers. Some of these individuals used film to record the behavior of the lesioned animals. The historical value of these films is undoubted. However, further analyses of these films by contemporary scientists might provide new information on primate motor function, or could be used to evaluate current theories. Accordingly, we have been collecting all the available films of CNS-lesioned monkeys, as well as the accompanying materials (eg., surgical protocols, histological slides, photographs). By far the largest collection is that of Binnay-Brown, which consists of about 3500 rolls of film of about 450 monkeys. In this presentation we primarily describe the Binnay-Brown collection. Binnay-Brown records of experimental primate lesion film collections. (eg, Woolsey, Bucy, Kuppers with Lawrence). We are anyone with an interest in using any one of these collections, as well as anyone knowing the whereabouts of additional collections, to contact us. Finally, videotapes of a few of the films from the Binnay-Brown collection are available for viewing at this meeting.
629.23 NEUROPATHWAYS MEDIATING CRAWLING BEHAVIOR IN THE MEDICINAL LEECH, Hirudo medicinalis. A. Bigder and W.B. Kristan Jr., Department of Biology, UCSD, La Jolla, CA 92030-0222.

Leeches produce two distinct locomotor behaviors: they can swim or crawl towards their targets. While swimming is a rather fixed oscillatory behavior which, once elicited, is hardly influenced by sensory information, crawling consists of many behavioral subcomponents that are strongly sensory-controlled. We are interested in (1) finding neuronal pathways participating in crawling and (2) the extent to which the nervous system recruits neuronal elements involved in other behaviors to produce crawling.

In freely behaving leeches, changes in crawling behavior were analyzed after cutting peripheral nerves and transecting the connectives. Deafferentation of several midbody segments (e.g., segments 8 through 18) does not hinder the conditioned head and tail sucker activity, but it decreases the velocity of body contractions. Transection of both connectives (between segments 11 and 12) blocks the propagation of the elongation wave, but does not prevent contraction of posterior segments and reattachment of the tail sucker, while animals with only one connective cut can still crawl in a coordinated manner. Thus, crawling seems to be mediated both by central information transfer through the connectives and partly by peripheral pathways.

In tethered crawling leeches, intra- and extracellular recordings were performed in midbody ganglia while the behavior was video-monitored simultaneously. The activity profiles of identified interneurons (e.g., 204, 208, S) were determined during crawling. The S-cell, for example, which forms a fast conducting pathway through the whole nerve cord is activated during the crawling phase. The S-cell also excites motoneurons which causes the inhibition of other circular motoneurons producing the elongation of the body during crawling. Supported by a Deutsche Forschungsgemeinschaft grant to AS and a USPHS grant MH43556 to WKB.


Our previous in vitro studies identified a limited region of the medullary ventrolateral medulla, the pre-Bötzinger Complex (pre-BöC), that contains neurons generating respiratory rhythm. Medullary slices (400 μm thick) containing this region generate oscillatory discharges in cranial nerves XII and/or IX (Smith et al., 1990; Smith et al., 1991, ibid), allowing analyses of mechanisms of rhythm generation and respiratory drive in an isolated and maintained respiratory circuit. To determine the role of EAAs in rhythmogenesis and synaptic transmission in this circuit, effects of local microinjections of NMDA (MnNMDA) and non-NMDA (CNQX) antagonists were studied. Unilateral injection of 20 μM CNQX solution (10 - 30 nl; 200 - 600 nl/min) into the pre-BöC produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections (50 nl) did not affect the size of maximum response but did affect rhythm, confirming site-specificity. Unilateral injection of 20 μM CNQX (2 - 25 nl) into the hypoglossal motor nucleus produced a dose-dependent decrease in burst amplitude and, at the site of maximum response, completely blocked the motor discharge of XII splanchnically, without blocking rhythmic discharge on the contralateral side. NMDA (5 μM) and non-NMDA (CNQX) antagonists were studied. In the pre-BöC region, the CNQX injections (50 nl) produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) remained effective. CNQX injections (50 nl) did not affect rhythm, confirming site-specificity. Unilateral injection of 20 μM CNQX (2 - 25 nl) into the hypoglossal motor nucleus produced a dose-dependent decrease in burst amplitude and, at the site of maximum response, completely blocked the motor discharge of XII splanchnically, without blocking rhythmic discharge on the contralateral side. NMDA (5 μM) and non-NMDA (CNQX) antagonists were studied. In the pre-BöC region, the CNQX injections (50 nl) produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections (50 nl) did not affect the size of maximum response but did affect rhythm, confirming site-specificity. Unilateral injection of 20 μM CNQX (2 - 25 nl) into the hypoglossal motor nucleus produced a dose-dependent decrease in burst amplitude and, at the site of maximum response, completely blocked the motor discharge of XII splanchnically, without blocking rhythmic discharge on the contralateral side. NMDA (5 μM) and non-NMDA (CNQX) antagonists were studied. In the pre-BöC region, the CNQX injections (50 nl) produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections (50 nl) did not affect the size of maximum response but did affect rhythm, confirming site-specificity.


Neurons of the medullary region contain a high density of glutamate receptors (GluR) which are activated by excitatory amino acids (EAAs). Glutamate and aspartate (Glu/Lys) injection (up to 25 nl; 200 - 600 nl/min) into the pre-BöC of rats during breathing (n = 15) produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections (50 nl) did not affect the size of maximum response but did affect rhythm, confirming site-specificity. Unilateral injection of 20 μM CNQX (2 - 25 nl) into the hypoglossal motor nucleus produced a dose-dependent decrease in burst amplitude and, at the site of maximum response, completely blocked the motor discharge of XII splanchnically, without blocking rhythmic discharge on the contralateral side. NMDA (5 μM) and non-NMDA (CNQX) antagonists were studied. In the pre-BöC region, the CNQX injections (50 nl) produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections (50 nl) did not affect the size of maximum response but did affect rhythm, confirming site-specificity. Unilateral injection of 20 μM CNQX (2 - 25 nl) into the hypoglossal motor nucleus produced a dose-dependent decrease in burst amplitude and, at the site of maximum response, completely blocked the motor discharge of XII splanchnically, without blocking rhythmic discharge on the contralateral side. NMDA (5 μM) and non-NMDA (CNQX) antagonists were studied. In the pre-BöC region, the CNQX injections (50 nl) produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections (50 nl) did not affect the size of maximum response but did affect rhythm, confirming site-specificity.

630.3 PLATEAU-POTENTIALS CONTRIBUTE TO THE GENERATION OF RHYTHMIC DEPOLARIZATIONS IN LOCUST FLIGHT INTERNEURONS. L.M. Ramires and K.G. Peper, Dept. of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7.

During flight in the locust the membrane potential of interneurons oscillates over a wide amplitude range (up to 50 mV). We found that in many interneurons (e.g., 308, 504, 514, 566 and 567) voltage-sensitive plateau potentials contribute to the generation of these large oscillations. Hyperpolarizing currents injected into these interneurons caused the amplitude of rhythmic depolarizations to drop suddenly by up to 50% in both intact and deafferented flying locusts. Plateau-potentials could be triggered by short depolarizing pulses and prematurely terminated by short hyperpolarizing pulses. A characteristic feature of these potentials was that they were rarely expressed in the quiescent locust. In the absence of flight activity most neurons displayed only passive membrane properties; in response to current injection. These data indicate that plateau-potentials are induced during flight activity. Induction of these active membrane properties during flight may depend on the natural release of EAAs from either the pre-BöC or other neural elements involved in other behaviors to produce crawling.

630.4 SEROTONIN-DEPENDENT PERIPHERAL SPIKE INITIATION ZONES IN THE AXON OF A MOTOR NEURON. I.M. Weimann, P. Meyrand*, and E. Marler, Biology Dept., Brandeis Univ., Waltham, MA 02245 and Neurobiologie, CNRS, Arcachon, France.

The lateral gastric (LG) neuron of the stomatogastric ganglion (STG) in the crab, Cancer borealis, makes inhibitory synaptic connections within the neuropil of the STG, and also projects to the periphery where it innervates muscles that control the movements of the gastric mill. The LG motor neuron has a spike initiation zone close to its neuropilar integrative regions. Spikes initiated here evoke IPSPs in LG follower neurons. During spontaneous gastric activity in semi-intact preparations (muscles attached) spikes can also be initiated at peripheral axonal sites (~ 2 cm from the LG soma) spikes can also be initiated at peripheral axonal sites (~ 2 cm from the LG soma). Peripherally initiated spikes propagate antidromically into the STG where they do not evoke IPSPs into LG follower neurons and also propagate to the periphery where it innervates muscles that control the movements of the gastric mill. The LG motor neuron has a spike initiation zone close to its neuropilar integrative regions. Spikes initiated here evoke IPSPs in LG follower neurons. During spontaneous gastric activity in semi-intact preparations (muscles attached) spikes can also be initiated at peripheral axonal sites (~ 2 cm from the LG soma). Peripherally initiated spikes propagate antidromically into the STG where they do not evoke IPSPs in LG follower neurons and also propagate to the periphery where it innervates muscles that control the movements of the gastric mill. The LG motor neuron has a spike initiation zone close to its neuropilar integrative regions. Spikes initiated here evoke IPSPs in LG follower neurons. During spontaneous gastric activity in semi-intact preparations (muscles attached) spikes can also be initiated at peripheral axonal sites (~ 2 cm from the LG soma). Peripherally initiated spikes propagate antidromically into the STG where they do not evoke IPSPs in LG follower neurons and also propagate to the periphery where it innervates muscles that control the movements of the gastric mill. The LG motor neuron has a spike initiation zone close to its neuropilar integrative regions. Spikes initiated here evoke IPSPs in LG follower neurons. During spontaneous gastric activity in semi-intact preparations (muscles attached) spikes can also be initiated at peripheral axonal sites (~ 2 cm from the LG soma). Peripherally initiated spikes propagate antidromically into the STG where they do not evoke IPSPs in LG follower neurons and also propagate to the periphery where it innervates muscles that control the movements of the gastric mill. The LG motor neuron has a spike initiation zone close to its neuropilar integrative regions. Spikes initiated here evoke IPSPs in LG follower neurons.
electrophysiological properties of lamprey spinal neurons were studied in the isolated spinal cord of adult lampreys. The neurons consist of two large regions representing the soma and primary neurite connected to an axon and a secondary process both of which have active Hodgkin-Huxley sodium and delayed rectifier conductances. The peripheral initiation zone located distally on the axon is modulated with an additional current that can produce a plateau potential resulting in a burst of action potentials.

As in the experimental preparation, we find the following features in the model: 1) There were generated and antidromic action potentials appear highly attenuated when recorded from the soma and have different shapes. 2) The peripheral zone can be activated by action potentials propagating outward from the primary initiation zone. Activation may require anywhere from one to a large number of spikes. Once activated, the peripheral initiation zone can generate a number of spikes or it can fire continuously. 3) As well as travelling down the axon, orthodromic spikes propagate down secondary processes to inhibit synapses. Antidromic spikes also propagate along the axon but they fail to propagate down secondary processes.

The results show that the postsynaptic effect of an action potential propagating along a neuron depends on the location of the spike initiation zone. Supported by MH64742.

630.7


Voltage-dependent changes in [Ca]i were examined in identified neurons of Cancer borealis, by using simultaneous electrical recording and high-speed imaging of Fura-2 fluorescence at 380 nm with a cooled CCD camera. The same somatic microelectrode was used to inject Fura-2, to inject current, and to record membrane potential. Sharp increases in [Ca]i, time-locked to depolarizing potentials, were interpreted as due to influx through voltage-gated calcium channels.

Our results extend those previously obtained using aminoncino III and a photodiode array (Graubard and Ross PNAS 82:5565-5569, 1985; Ross and Graubard PNAS 80:1679-1683, 1983). Use of Fura-2 and the camera enabled us to estimate the magnitude of the changes in [Ca]i and to identify with greater spatial precision the sites of [Ca]i increase.

For all cells, bursts of intrasomatically evoked action potentials caused calcium entry into the soma and neuropil with distinctly higher levels in a small region of the main process (pre-axon) near the edge of the neuropil just before the axon enters a nerve. Cells with more than one axon had a "hot spot" on each pre-axon. During the spontaneous oscillations and spike bursts of the pyloric rhythm, oscillations of [Ca]i were observed with the highest amplitudes at the "hot spots." In TXA saline, intrasomatic stimulation caused increases in calcium which were largest in the soma and decreased smoothly in the neuropil; no "hot spots" were seen.

We suggest that 1) during spontaneous bursting the mean variation in [Ca]i in the neuropil is small; 2) most spike-evoked synaptic transmission comes from the subset of neurites closest to the "hot spots"; 3) the calcium channel distribution along the largest neurites is roughly uniform; and 4) there are few calcium-permeant ion channels in the axon.

Supported by N.I.H. Grants NS16295(WNR), NS25505(KG).

630.8

Regional distribution of the fine neurites of identified neurons of the crab stomatogastric ganglion. D. Baldwin and K. Graubard, Dept. of Zoology, University of Washington, Seattle, WA 98195.

The stomatogastric neuropil of crabs and lobsters can be divided into a central core containing the largest neurites, an intermediate region containing a mix of intermediate and fine neurites, and a peripheral neuropil containing mostly fine neurites. Neurons of the STG participate in two major motor patterns - peristaltic and pyloric - which are controlled by two different neural circuits. We have examined the regions of fine neurites in neurons that participate in both or one of these circuits to determine if the circuitry is organized in a regional manner.

Identified stomatogastric neurons of Cancer borealis were injected with Lucifer Yellow, fixed and examined as whole mounts with a confocal microscope. In some cases, another dye was injected into a second neuron. For each cell, the neuropil was divided into radial arcs and the serial images scored based on the presence of fine neurites within each arc. This representation of the three-dimensional spread of the fine neurites of each neuron was compared with those of the other neurons. Examinations of PD and LP, purely pyloric neurons, and VP, a mixed pyloric/gastric neurone, showed no differences in the distribution of regions of fine neurites.

Supported by N.I.H. Grants NS16579 and NS25505 to K. Graubard.

630.10

The effects of strychnine on fictive swimming in the lamprey. D.R. McPherson and J.T. Buchanan, Department of Biology, Marquette University, Milwaukee, WI 53233.

Previous studies demonstrated that strychnine speeds fictive swimming in the isolated lamprey spinal cord, presumably by reducing glycnergic inhibitory synapses involved in rhythmogenesis. In contrast to these experimental results, computer simulations of the lamprey locomotor network model display slowing of cycle rate when all inhibitory connections are reduced. The effect is dependent on cell type: reduction of only CC inhibitory neurons had no effect, while reduction of all neurons (CC and LINs) upon CC interneurons, while bicuculline has no effect.

The effect of strychnine on fictive swimming has therefore been re-examined. Confirming earlier reports, strychnine accelerates the swim rate in adult silver lampreys (Ichthyomyzon americanus), but in adult sea lampreys (Petrogaster macrocephalus), low concentrations (0.5μM) of strychnine produce a slight speeding, while higher concentrations (2μM) elicit extremely slow alternating bursting (10% of control rate). The slow rate appears to developed as a progressive fusing of fast bursts into longer ones.

Thus, there appears to be a species difference in the response to strychnine during fictive swimming, these results may indicate a need to revise the proposed locomotor network.
Effects of Dextemazine (D) and Quisqualate (Q) Lesions of the Nucleus Basalis Magnocellularis (NBm) on Cortical EEG. R.J. Rudek, M. Decker, J.A. Rudek, M.C. Rudek, J. Little, and R. Little. National Institute on Drug Abuse, Research Triangle Park, NC. 27709.

Lesions of the NBm produce deficits in various learning and memory tasks. The several lesion methods used to generate damage result in similar depletions of the cholinergic marker choline acetyltransferase (ChAT), but do not produce similar behavioral effects. For example, D lesions impair performance in the standard Morris water maze task, whereas Q lesions do not. The purpose of this study was to compare the effects of these two NBm lesions on cortical EEG. Bilateral lesions were made with either D (0.06 mg/kg) or Q (0.12 mg/kg) in long-term cathode coordinates (mm): AP, -6.0; ML, 5.5; VS, -5.0. Frontal-posterior SEI was recorded bilaterally and FFT calculated. Power spectrum analysis is expressed as percent change from the non-lesioned hemisphere. D-lesioned rats had a significant 5% (p<0.05) change of alpha (8-13 Hz) power in the ipsilateral frontal-posterior cortex. QW activity in frontal-posterior cortex was decreased 5% (p>0.05) with both Q and D lesions, respectively. These data indicate that D and Q have differential effects on brain injury and may be distinguished on the basis of cortical electrical activity.


Cortical inputs potentiate a hyperpolarization-activated slow thalamic rhythm (0.5-4 Hz) and synchronize oscillator relay neurons that are otherwise uncoupled (Steriade et al., this meeting). Since these facilitating/synchronizing actions were exerted by volleys arising in cortical areas that were not directly related to the explored thalamic nucleus, we hypothesized that they were due to the conjunction properties of the cortical-driven GABAergic thalamocortical connections displayed the slow oscillation as their membrane potential progressively hyperpolarizes during late stages of sleep. We postulate a hyperpolarizing blockage of thalamic cells with the deepening of quiet sleep, which would account for spindling during early stages and delta waves during late stages. By contrast, brainstem and/or sensory depolarizing inputs would explain the delta blockage at the transition from slow-wave sleep to either arousal or REM sleep. Supported by MRC grant MT-3689.


An intrinsic oscillation in the frequency range of sleep EEG delta waves (0.5-4 Hz) has been studied in cat thalamocortical neurons. Antidromically identified cortical-projecting cells have been recorded intra- and extracellularly in most major thalamic nuclei under urethane or nitrous oxide-halothane anesthesia. About 80% of cells with intact cortical connections displayed the slow (delta) oscillation as their membrane potential was between -60 and -80 mV by DC. The oscillation consisted of rhythmic low-threshold spikes and after-hyperpolarizations, and it was not dependent upon the occurrence of fast action potentials. This rhythm, involving an interplay between the hyperpolarization-activated cation current (ih) and the low-threshold transient Ca+ current (Ih), was similar to that reported by McCormick & Pape (1990) in a subpopulation of dLG cells in vitro. The oscillatory activity was spontaneously present in virtually all neurons anatomically or functionally disconnected from related cortical areas (Vm > -65 mV), in which case it could be blocked by DC depolarization. Under these experimental conditions focal delta waves built-up during epochs with synchronized rhythmic multiunitary firing. Only anterior thalamic neurons did not display a delta oscillatory capability.

We hypothesize that this intrinsic phenomenon contributes to the switching of thalamocortical neurons toward slow delta waves, as their membrane potential progressively hyperpolarizes during late stages of sleep. Supported by MRC grant MT-3689.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991
The calcium binding proteins, parvalbumin (PV) and calbindin D-28k (CB), are present in morphologically distinct subpopulations of nonpyramidal neurons in monkey neocortex (PNAS 83:2093; Exp Brain Res 76:476, 1989; JCN 290:599, 1990). In this study, we used immunohistochemical techniques to characterize the distribution of immunoreactivity for a related calcium binding protein, calreitin (CR), in cerebral cortex of infant and adult rhesus (Macaca mulatta). Observations in basal forebrain (1-Cysk), hypothalamus, ventral tegmental area, raphe and locus coeruleus revealed labelled neurons predominantly ipsilateral to the injection site, with varying degrees of contralateral labelling. Overall, the raphe showed the highest percentage of contralaterally labelled neurons (35% of all labelled neurons), while the basal forebrain cholinergic neurons showed the lowest percentage (less than 1%). In the locus coeruleus, contralateral labelling was considerably higher after hippocampal injections than after dorsal raphe or other non-cholinergic nuclei examined. In this study did not show consistent differences of laterality in their projections to hippocampal cortex. The layer of extrahippocampal corticocortical projections thus varies from one nucleus to another and, in the case of the locus coeruleus, also as a function of the target projection site.

**361.11** DISTRIBUTION OF CALREITIN IMMUNOREACTIVITY IN MONKEY CEREBRAL CORTEX. D.A. Lewis, I.S. Lund, M. Alk, and D.M. Jacobowitz. Dept. of Psychiatry and Behavior, Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15261 and Ab. of Clinical Science, NIH, Bethesda, MD 20892.

Immunohistochemical techniques were developed to characterize the distribution of immunoreactivity for a related calcium binding protein, calretinin (CR), in cerebral cortex of infant and adult rhesus (Macaca mulatta). Observations in basal forebrain (1-Cysk), hypothalamus, ventral tegmental area, raphe and locus coeruleus revealed labelled neurons predominantly ipsilateral to the injection site, with varying degrees of contralateral labelling. Overall, the raphe showed the highest percentage of contralaterally labelled neurons (35% of all labelled neurons), while the basal forebrain cholinergic neurons showed the lowest percentage (less than 1%). In the locus coeruleus, contralateral labelling was considerably higher after hippocampal injections than after dorsal raphe or other non-cholinergic nuclei examined. In this study did not show consistent differences of laterality in their projections to hippocampal cortex. The layer of extrahippocampal corticocortical projections thus varies from one nucleus to another and, in the case of the locus coeruleus, also as a function of the target projection site.
631.13

**CHELOCYSTOKININ-IMMUNOREACTIVE NEURONS IN MONKEY VENTRAL MECENSEPHALON PROJECT TO PREFRONTAL CORTEX BUT DO NOT INHIBIT DOPAMINERGIC NEURON ACTIVITY.** K. Okh and D.A. Louis. Dept. of Behavioral Neuroscience and Psychahl. Univ. of Pittsburgh, Pittsburgh, PA, 15213.

In rodents, the CCK-immunoreactive (IR) neurons in the ventral mesencephalon (VMC) are known to contain tyrosine hydroxylase (TH) and to project to prefrontal cortex (J Comp Neurol 279:397, 1989/90). In primates, some CCK-IR neurons do not contain TH (PNAS 67:8427, 1990). We have observed that the distribution of these CCK-positive/TH-negative cells in monkey (Macaca fascicularis) VMC follows a rostroradial gradient. For example, at the dome levels, CCK-IR cells are present medially in an area that is almost totally devoid of TH-positive neurons. In more caudal regions of the VMC, the location of CCK-containing neurons overlaps with the distribution of TH-IR cells, although CCK-positive/TH-negative neurons are still observed. We have previously found that CCK-containing cells in monkey VMC project to prefrontal cortex; however, it is not known if these neurons belong to the class of CCK-positive/TH-negative neurons. Using combined retrograde transport techniques and immunohistochemistry, we found that CCK-containing cells in the VMC which project to prefrontal cortex do not contain TH. These observations were confirmed in double labeling studies in prefrontal cortex which also demonstrated a lack of colocalization of CCK and TH in neighboring terminal fields. We further find that a population of CCK-containing neurons in the VMC has the capacity, independent of dopaminergic, to directly influence prefrontal cortical function.

631.15


The prefrontal cortex (PFC) contains neural circuits engaged in working memory and it is of interest to determine how the cortical cholinergic innervation may influence this circuitry. Therefore, cholinergic synapses in areas 9 and 46 of the PFC of three adult threes and two cynomolgus monkeys were visualized using a monoclonal antibody against choline acetyltransferase (CHAT, provided by Dr B. Wainer). In addition, a double labeling electron microscopic (EM) method was used in which immunoperoxidase reactive cholinergic synapses were visualized in contact with immunoperoxidase labeled GABAergic nonpyramidal neurons. Cholinergic synapses in contact with morphologically identified pyramidal neurons were also characterized at the EM level. Cholinergic synapses were found in all layers of the PFC and all synaptic contacts were symmetric. Chat positive profiles were in synaptic contact with both pyramidal and GABAergic neurons. The predominance postsynaptic targets on pyramidal neuronal terminals and apical and basal dendritic shafts and less frequently dendritic spines. Cholinergic terminations on GABA-immunoreactive neurons were restricted to dendritic shafts. These results are the first demonstration of cholinergic synapses in the primate association cortex and are in accord with physiological data showing that cholinergic axons influence principal neurons in the cortex both by direct excitation and indirectly via feedback inhibitions by GABAergic interneurons. These findings elucidate an anatomical substrate for the cholinergic modulation of corticofugal function. Supported by MH 44866 and NS 26068.

631.17


An analysis of Nissl, myelina, ACNE and immunohistochemical staining patterns of the orbital and rostral insula of Macaca fascicularis indicates that these regions encompass 17 distinct areas, as seen in the following unfolded map:

<table>
<thead>
<tr>
<th>Area</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anterior agranular area</td>
</tr>
<tr>
<td>2</td>
<td>Lateral agranular area</td>
</tr>
<tr>
<td>3</td>
<td>Medial agranular area</td>
</tr>
<tr>
<td>4</td>
<td>Ventrolateral agranular area</td>
</tr>
<tr>
<td>5</td>
<td>Ventromedial agranular area</td>
</tr>
</tbody>
</table>

This analysis is based on the study of serial sections of the cerebral cortex and thalamus in the rhesus monkey. The ventral agranular area (VAG) receives afferents from the amygdala and the entorhinal cortex. The lateral agranular area (LAG) receives afferents from the piriform cortex and the entorhinal cortex. The medial agranular area (MAG) receives afferents from the entorhinal cortex and the lateral entorhinal cortex. The ventrolateral agranular area (VLAG) receives afferents from the entorhinal cortex and the lateral entorhinal cortex. The ventromedial agranular area (VMAG) receives afferents from the entorhinal cortex and the lateral entorhinal cortex. The anterior agranular area (AAG) receives afferents from the amygdala and the entorhinal cortex. The lateral agranular area (LAG) receives afferents from the piriform cortex and the entorhinal cortex. The medial agranular area (MAG) receives afferents from the entorhinal cortex and the lateral entorhinal cortex. The ventrolateral agranular area (VLAG) receives afferents from the entorhinal cortex and the lateral entorhinal cortex. The ventromedial agranular area (VMAG) receives afferents from the entorhinal cortex and the lateral entorhinal cortex.

631.14

**DOPAMINERGIC (DA) AND MORPHOGENETIC (NA) MODULATION OF THE RAT FRONTAL CORTEX.** E. Canedo (1), M. Dominguez (1), I. Yuste (2), G. Valverde (2), M. Fernández (2), J. M. Varela (2). (1) CERECOR UMA 1121, (2) UAB 40100, Barcelona, Spain.

In this study we compared across adjacent sections the distribution of NA and DA innervation of the rat frontal cortex, as revealed with respectively polyclonal and monoclonal antibodies directed against NA- or DA- glutatione-pheynyl conjugatates (Geffard et al. Brain res., 1986, 363:395-400; Jones et al. Brain res., 1987, 4:397-494). Our results confirmed and extended previous results obtained with different techniques and emphasized neuroanatomical differences between DA and NA innervation. Regional differences included a medio-frontal trend, in which density of DA and NA fibers was lower than that of area (PL) and ventro-lateral orbital area in which density of DA fibers is weak whereas NA fibers are numerous. Laminar differences were observed in PL, with tangential fibers mostly run in the middle part of layer I, whereas DA tangential fibers are mostly in the outer part of layer I. These differences suggest morpho-functional differences for NA and DA in frontal areas. Using a combined quantitative study of density of NA and DA fibers will be further attempted.

631.16

**SINGLE UNIT ACTIVITY IN PREFRONTAL CORTEX AND MEDIAL DORSAL THALAMUS IN THE RAT: BEHAVIORAL RESPONSIVENESS AND EFFECTS OF ETHANOL.** Kondak, A.E., and Chapin, J.K., Hahnemann University, Philadelphia, PA, 19102.

Using chronically implanted microcircuit electrodes, it is possible to obtain repeated recordings simultaneously from multiple neurons in awake, behaving animals. In the present experiments, multiple bundles of 24-46 microcircuit electrodes were chronically implanted in MD thalamus and various sites in medial frontal and prefrontal cortices of rats, 250-300 g. Prefrontal and frontal cortex play a role in planning and organization of behavior, suppression of competing impulses, and in short-term task-related memory. These areas may be important sites for ethanol action, especially its disinhibiting and rewarding properties. Initially, animals were trained to walk on a treadmill with an intermittent cycle. Cessation of treadmill movement was signalled by a fixed time interval or a tone cue. In subsequent experiments, rats were trained to press a lever to terminate treadmill movement. A population of CCK-containing neurons in the VM of the rat core: axons which influence principal neurons in the cortex both by direct excitation and indirectly via feedback inhibitions by GABAergic interneurons. These findings elucidate an anatomical substrate for the cholinergic modulation of the whole animal. In the present study examined the physiological correlates of severe neglect through the use of [14C] 2-deoxyglucose methodologies. The functional correlates of neglect were examined in rats which received unilateral lesions of either the left AGm or the left lateral agranular cortex (AGL). Unlesioned controls were also examined. The physiological procedures were performed 4-6 days postop., after the rats were tested for the presence of neglect. Glucose utilization was determined by WD-1200 microdialysis. The results indicated that glucose utilization was significantly reduced ipsilaterally in the posterior striatum, the superior colliculus, and thalamus in the nuclei MD and ventrals lateralis. In the cortex glucose metabolism was significantly reduced in the AGm and medial agranular cortices. These results generally support the pattern of glucose utilization found following physiological studies of frontal neglect in primates.

Funded by LEQSf grant RD-A-17 to JVC.

631.18


Our previous work has demonstrated that unilateral destruction of the medial agranular cortex (AGm), in rodents produces severe multimodal neglect. The present study examined the physiological correlates of severe neglect through the use of [14C]- 2-deoxyglucose methodologies.

The functional correlates of neglect were examined in rats which received unilateral lesions of either the left AGm or the left lateral agranular cortex (AGL). Unlesioned controls were also examined. The physiological procedures were performed 4-6 days postop., after the rats were tested for the presence of neglect. Glucose utilization was determined by WD-1200 microdialysis. The results indicated that glucose utilization was significantly altered in rats with severe neglect produced by unilateral AGm lesions. Glucose utilization was significantly reduced ipsilaterally in the posterior striatum, the superior colliculus, and thalamus in the nuclei MD and ventrals laterialis. In the cortex glucose metabolism was significantly reduced in AGm and AGL. These results generally support the pattern of glucose utilization found following physiological studies of frontal neglect in primates.
631.19

Our previous anatomical work has demonstrated that a posterior area of parietal cortex lying just lateral to the cingulum bundle is reciprocally connected with mediodorsal thalamus and ventrolateral orbital cortex. Unilateral lesions of either of these latter two areas produces contralateral multimodal neglect. We now report that severe contralateral neglect also results from unilateral lesions selective to this posterior parietal area.

To delineate the connectivity of this posterior parietal field, rats received microparticles of three different fluorescent tracers, spaced in the anteroposterior or mediolateral direction. Within the a-p series, all cases had cortical labeling in mediodorsal and orbital cortex. Thalamic labeling involved the ventral posterolateral, lateral dorsal/lateral posterior, lateral and mediodorsal geniculate nuclei. More rostral injections produced heavier labeling in ptf and zona incerta, typical of a somatic sensory cortex while more caudal injections resulted in the reverse, typical of visual areas; all three injection locations yielded labeling in both. Within the m-d series, more mediodorsal injections produced thalamic labeling which included the anterior thalamus, typical of reticulothalamic cortex. Middle and lateral placements did not differ in thalamic and cortical labeling patterns. We conclude that a discrete posterior parietal area exists in the rat, based on the behavioral findings related to multimodal neglect, and on a combination of cortical and thalamic labeling patterns.

Funded by Univ. of Florida College of Veterinary Medicine (RUR) and Dept. of Neurosurgery (HCC), and grant LRESF RD-A-17 (VKR, JVC).

631.21

The anterior ectosylvian sulcus (AES) has been considered a polysensory/multisensory cortical region where convergence of different sensory inputs onto individual neurons occurs. However, three different modality-specific regions have been identified in the AES, suggesting that the degree of multisensory convergence may be less extensive than expected. Therefore, the AES cortex of 9 cats was examined to determine the degree of multisensory convergence. The vast majority of AES neurons encountered (93%, 499/537) of sensory neurons encountered proved to be unimodal and visual, auditory, and somatosensory neurons were segregated from one another: somatosensory in the rostral dorsal bank, visual on the caudal ventral bank, and auditory on the caudal bank which corresponded closely to the identified regions of SIV, EVA and Field AES. Multisensory neurons constituted only about 7% (38/537) of the population, and were found primarily near the borders of adjoining modality-specific fields. Given the paucity of multisensory convergence here it seems inappropriate to retain the designation 'polysensory' cortex. Rather, the AES is best regarded as a composite of three different unimodal areas flanked by strips where sensory convergence sometimes occurs.

Supported by RNS 8719234, NS 22543, NS 08902.

631.22

The rules by which neurons integrate information from different senses have been identified in the superior colliculus (Meredith and Stein, J.Neurophysiol 56:660 ’86) and their role in multisensory orientation behaviors have been demonstrated (Stein et al., J.Cognit.Neurosci 1:12’89). However, their general applicability across the neuraxis is not known. The present experiments examined this issue in single neurons in the cortex of cat anterior ectosylvian sulcus. Among the multisensory neurons encountered (n=24/156), all combinations of visual, auditory and somatosensory inputs were identified. When stimuli from different modalities were combined, the responses were dramatically enhanced or depressed. These interactions were often multiplicative and were dependent on the same factors evident in the superior colliculus: receptive fields for the different modalities were spatially aligned; combined stimuli presented within their respective receptive fields evoked response enhancement; a stimulus falling outside its receptive field either depressed or had no effect on an effective stimulus from another modality; the temporal window for integration far exceeded the duration of the unimodal discharge trains; maximal levels of enhancement occur when stimulus onset is adjusted to overlap the peak periods of the discharge trains. These data indicate that the rules governing multisensory integration are independent of structure and apply to neural and behavioral processes throughout the CNS. Supported by NS 25243.

632.1

The central cholinergic system has been implicated in learning and memory processes. 1-125-4-Iododexetimide (1-125-DEX) is a high affinity muscarinic cholinergic receptor (mAChR) antagonist which has been used to study mAChR distribution and imagery. This study examines the effects of behavioral training on mAChR distribution in the brain of the rat. Three experimental groups were trained on the Morris water maze learning task: groups A, B, and C. Group A was trained on a 90° turn; group B was trained on a 180° turn; and group C was trained on a 270° turn. The groups were trained for 10 days. After the training period, the rats underwent a two-choice spatial discrimination task. The rats were placed into two separate arms of the maze and allowed to explore for 10 minutes. The arms were interchanged from day to day. The rats then underwent a spontaneous spatial extinction task. The rats were placed into one arm of the maze and allowed to explore for 10 minutes. The arm was then changed and the rats were placed back into the new arm for another 10 minutes. The rats were then sacrificed and the brains were sectioned and autoradiographed. The brains were then sliced and the sections were autoradiographed. The sections were then cut into 40 µm thick slices and the brains were then autoradiographed. The autoradiograms were then analyzed for mAChR binding. The results of this study showed that there was a significant increase in mAChR binding in the hippocampus and the thalamus of the rats that were trained on the Morris water maze task. The results of this study suggest that there may be a relationship between learning and memory and mAChR binding.
632.3  
MORRIS WATER MAZE PERFORMANCE IN 3 WEEK OLD RATS - SEX DIFFERENCES AND HORMONE EFFECTS.  
R.L. Roof, Dept. of Psychology, University of Nevada, Reno, NV 89557.  

It has been demonstrated that adult male rats outperform females on spatial tasks, including the radial arm maze and the Morris water maze. In addition, this sex difference can be modified with estradiol administration to testosterone. Testosterone treated females perform better than control females, and in some cases, as control males. In human studies of spatial ability, gender differences are not often found in prepubertal populations. It is not known when sex differences for spatial tasks first appear in rats, and whether the administered hormones exert their effects early in life or in a delayed manner. In order to determine whether such sex differences exist in very young rats, male and female rats, both testosterone treated and controls, were trained and tested in the Morris water maze at a young age.

Sprague-Dawley rats were treated with 250 ug testosterone propionate on postnatal days 2 and 4. Controls were given only the oil vehicle. At three weeks of age, these rats were trained and tested on 10 consecutive days in a Morris water maze. The task consisted of finding and remembering the location of a submerged hidden platform in milk water. Sex differences and effects of the testosterone are described in terms of time required to find a platform over trials, as well as time spent in each quadrant on probe trials.

The understanding of the time course of development of sex differences in spatial performance of rats and the underlying physiological bases for these sex differences are to be determined.

632.5  
EFFECTS OF BENZODIAZEPINE RECEPTOR LIGANDS ON THE PERFORMANCE OF AN OPERANT DELAYED MATCHING TO POSITION TASK IN RATS.  
Department of Psychiatry, Schering AG, Berlin, Federal Republic of Germany.  

The effects of a series of benzodiazepine (BZD) receptor ligands, ranging from full agonists through antagonists to partial inverse agonists on short term working memory in the rat were investigated. The behavioral paradigm used was discrete trial, operand delayed matching to position, with delays of 0.5, 1.5 and 30 s. These delays generated an orderly 'forgetting' curve in control rats. Diazepam and hirzapem (BZD agonists) both produced a delay dependent impairment in matching accuracy at certain doses, but delay independent effects at higher doses, which were accompanied by behavioral indices of sedation, including lengthening of reaction times. The BDZ receptor partial inverse agonist FG 7142 also produced a delay dependent impairment in matching accuracy, but also lengthened reaction times. Taken together, these results suggest that as optical level of cortical and subcortical structures is necessary for accurate performance of delayed matching to position in rats, and that both increases and decreases in GABA function, effected through BDZ receptor ligands, can impair performance.

632.6  
AMNESIC EFFECTS OF CHLORDIAZEPoxide IN THE RADIAL ARM MAZE.  
I.S. Shumsky and L. Lucki.  
Departments of Pharmacology and Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.  

The benzodiazepine (BZ) chlordiazepoxide (CDP) produces amnesia in humans but has been reported to produce variable effects in rats on performance in the radial arm maze in tasks used to measure memory. Chronic pre-treatment administration of saline or CDP (5, 10, 20 mg/kg, ip) to food-deprived male Sprague-Dawley rats produced impaired performance of memory over a 21-day training period. The number of trials required to attain a criterion of 2 or fewer errors on 3 consecutive days and the percentage of animals reaching that criterion was: saline, 7.4 ± 1.2 (100%); 5 mg/kg, 10.0 ± 1.6 (97.5%); 10 mg/kg, 11.0 ± 2.9 (30%); and 20 mg/kg, 12.0 ± 1.0 (25%). Measures of accuracy (number of errors and task efficiency) were impaired by 10 and 20 mg/kg CDP. Measures of learning (ratio of errors in the acquisition phase to the total errors corrected for the delay phase) were unaltered at 10 mg/kg CDP but reduced at 20 mg/kg CDP. In a second experiment, pretreatment with the BZ receptor antagonist flumazenil (5 mg/kg, ip) reversed the impaired performance of 10 mg/kg CDP, although flumazenil produced no effect on its own. These results are in contrast to the effects in highly trained animals in which CDP (0.3-18 mg/kg, ip) produced no significant alteration in measures of accuracy and a progressive impairment of motor responsiveness in a cumulative dose-effect curve. Therefore, tasks of acquisition appear to be more sensitive than previously acquired tasks to disruption by CDP. Supported by DA 05186.
362.9

SPATIAL LEARNING IN THE MORRIS WATER TASK AFTER MEDIAL FRONTAL CORTEX LESIONS IN THE RAT: EFFECTS OF DIAZEPAM. M. S. Weaver, T. Schallert and D. C. James. Inst. for Neuroscience, Univ. Texas, Austin, TX 78713.

Diazepam chronically disrupts recovery from sensorimotor asymmetries caused by unilateral anterior medial cortex lesions in animals trained continuously during a sensitive 12-24 hour postoperative period (Schallert, Hernandez & Barth, 1986).

Diazepam appears to exaggerate secondary degeneration in the striatum which may contribute to the disruption of recovery (Jones & Sarter, 1991). In the present study, bilateral medial frontal cortex lesions significantly impaired spatial learning ability in Whishaw’s learning-set version of the Morris water task. Unexpectedly, recovery was greatly facilitated in animals trained secondarily with diazepam.

Previous results indicate that diazepam may allow sparing in the CA1 and CA2 regions of the hippocampus. Such a finding suggests that spatial deficits produced by medial frontal cortex lesions may result from secondary damage at the hippocampal level.

Depending on the neurological test, diazepam may retard or promote recovery function after medial frontal cortical lesions. (Supported by NS-23944 and AA-07471).

362.10

EFFECTS OF PERIPHERAL AND CENTRAL INJECTIONS OF GABA AGONISTS ON SPONTANEOUS ALTERNATION PERFORMANCE. C. M. GREY, W. K. LAM*, and P. E. GOLD. Neuroscience Graduate Program and Department of Psychology, U. Virginia, Charlottesville, VA 22903.

Peripheral, intraventricular and medial septal injections of glucose attenuate memory deficits caused by opiate agonists and cholinergic antagonists. GABA neurons are associated with cholinergic-opioid interactions in the medial septum, and injection of GABA agonists into the medial septum impairs memory (Chrobak et al., 1989; Broni et al., 1990). The present experiments examined the efficacy of peripheral and medial septal injections of glucose in attenuating spontaneous alternation deficits produced by GABA agonists.

A. Mice injected (IP) with either a GABA-A receptor agonist, muscimol (0.5 mg/kg) or a GABA-B receptor agonist, baclofen (5.0 mg/kg) 30 min before spontaneous alternation tests exhibited significantly impaired alternation performance (percent alternation scores: 62.7 ± 4.2% vs. 76.7 ± 6.2%, respectively, vs. saline 69.7 ± 1.4). This deficit was not reversed by co-administration (IP) of glucose (100 or 300 mg/kg). Muscimol injections directly into the medial septum (1 nmol/0.5 μl over 1 minute) impaired alternation performance (43.2 ± 7 vs saline 69.0 ± 3.8); this deficit was not attenuated by peripheral (100 mg/kg) or septal (3.0 mg/kg/0.5 μl over 1 minute) injections of glucose.

With parallel findings that glucose does not enhance memory in rats with lesions of the medial septum, the results suggest that glucose effects on memory require the participation of GABA neurons contained in the medial septum, which may act through the amygdala by processing via regulation of opioid and cholinergic systems. [Supported by ONR N0001499-J-1216, NIA (AG 07648), and NSF (BNS-901229)].

362.11


The triazole MDL 26,479 has been shown to displace the cortical binding of Ro15-1788, to reduce the effects of GABA on the activity of Purkinje cells, and to facilitate LTP (Dudley et al. 1990; Sorensen et al., 1990a). In contrast to benzodiazepine receptor inverse agonists (BZRIA), however, MDL 26,479 does not exhibit convulsive effects (Sorensen et al. 1990b), suggesting BZR-selective influences interact with GABAergic influences within the amygdala in regulating memory storage.

MDL 26,479 (5 mg/kg; i.p.; daily: 60 min pretest). in comparison to sham-lesioned controls, learning in lesioned rats was drastically impaired. MDL 26,479 did not improve the performance of sham-lesioned animals. However, MDL 26,479-treated, lesioned rats acquired the task as rapidly and accurately as controls. While the extent to which the behavioral effects of the ibotenic acid-induced lesion depended on the destruction of cholinergic neurons remains unclear, we speculate that the beneficial behavioral effects of MDL 26,479 were associated with cortical hypochoolinergic functions.

362.13

INTERACTION OF NORADRENERGIC, MUSCARINIC AND GABAERGIC SYSTEMS IN THE AMYGDALA IN REGULATING MEMORY STORAGE. I.B. Introini-Collison, C. Dalmau*, T. Salinas* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychology, University of California, Irvine, CA 92717.

Previous results from our laboratory have indicated that several neurochemical systems in the amygdala are involved in the modulation of memory storage. These experiments examined the interaction of the amygdala noradrenergic system with muscarinic and GABAergic systems. Postulating that amygdala administration of clonopitrol produced a dose-dependent enhancement of retention of an inhibitory avoidance task. The enhancing effects of clonopitrol (3.0 ng) were completely reversed by concurrent administration of atropine (1.0 μg), which by itself had no effect on retention.

In addition, atropine administration of a low dose of propranolol (0.3 μg), which alone did not affect retention, shifted the dose-response curve of the GABAergic receptor agonist muscimol (0.01, 0.03 and 0.1 ng) toward the left, i.e. lower doses of muscimol were necessary in the presence of propranolol to obtain similar effects.

These findings are consistent with other evidence suggesting that noradrenergic influences interact with GABAergic influences within the amygdala in regulating memory storage and that these influences are mediated through effects involving a muscarinic cholinergic mechanism.

Supported by UPHS MH12526 from NIMH and NIDA and ONR N00014-90-J-1626 (to JLM).
632.15


DuP 996 (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) enhances K+-stimulated release of several neurotransmitters in rat hippocampal CA1 neurons. DuP 996 is essentially inactive in binding to muscarinic and nicotinic receptors and in inhibiting cholinesterase activity. Behaviorally, DuP 996 was compared to Physostigmine (PH), and Tetrahydroaminoacridine (THA) for its ability to: 1) prevent hypoxia-induced loss in rats. DuP 996 increased the number of presynaptic vesicles in CA1 neurons, and 2) prevent hypoxia-induced disruption of visual acuity in squirrel monkeys. In these tests DuP 996 was found to be superior in that it either produced greater efficacy, was more reliable, and/or had a broader therapeutic window with a better safety margin (Table 1).

Table 1: Activity in mg/kg

<table>
<thead>
<tr>
<th>Activity</th>
<th>DuP 996</th>
<th>THA</th>
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<tbody>
<tr>
<td>Test</td>
<td></td>
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<tr>
<td>Hypoxia Passive</td>
<td>0.01-0.1</td>
<td>0.3-5.0</td>
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<tr>
<td>Avoidance (AV)</td>
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<tr>
<td>Lever Press Acquisition (LPA)</td>
<td>0.1-1.0</td>
<td>IA*</td>
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<tr>
<td>Monkey Visual Recognition (MVR)</td>
<td>0.3-0.6</td>
<td>IA</td>
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<td>Tremor (T)</td>
<td>10</td>
<td>3</td>
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<td>Monkey (MO)</td>
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*IA = inactive

These results suggest DuP 996 may have therapeutic value in the treatment of diseases which result in cognitive dysfunction.

632.19


DuP 996 (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) enhances K+-stimulated release of several neurotransmitters in various regions of rat brain in vitro. To explore its mechanism of action, conventional intracellular recording techniques were used to study the effects of DuP 996 on electrophysiological characteristics of CA1 pyramidal cells in rat hippocampal slices. DuP 996 (5-100 μM) enhanced the slow cholinergic epsp, reduced spike frequency adaptation (SFA), delayed repolarization of action potentials elicited by brief, threshold-level depolarizing pulses and increased the frequency of spontaneous action potentials, but had no consistent effect on resting membrane potential, input resistance, or inward rectification in response to hyperpolarizing current pulses. The effect of DuP 996 on epsp was blocked by atropine. The effect on SFA was mimicked by 100 mM d-tubocurarine. DuP 996 blocked both IA and IK nonselectively in a concentration-dependent manner above 30 μM; the half maximal inhibitory concentration (IC50) was approximately 1-2 μM.

632.18


Using EEG as a measure of brain electrical activity we examined the effects of DuP 996 on the EEG of 3 animal species: rat, rabbit and dog. Frontal-parietal, bipolar recordings were made from rats (9-13 Hz), rabbits (9-13 Hz) and multipole (16 electrodes) recordings were made in the rabbit for topographic analysis. EEG activity was collected online (125 Hz) and transformed into power spectra via a Fast Fourier transform. The results were then grouped into the following EEG bands (theta, 4-7 Hz; alpha, 8-13 Hz; beta, 13-35 Hz; and gamma, 35-100 Hz) and analyzed for changes in the power content of each EEG band. DuP 996, a Compound with Cognitive Enhancing Properties.

632.20


DuP 996 (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) enhances K+-stimulated release of several neurotransmitters from rat neocortical neurons. DuP 996 enhanced the slow cholinergic epsp, increased the number of presynaptic vesicles in CA1 neurons, and prevented hypoxia-induced disruption of visual acuity in squirrel monkeys. In these tests DuP 996 was found to be superior in that it either produced greater efficacy, was more reliable, and/or had a broader therapeutic window with a better safety margin (Table 1).

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*IA = inactive

These results suggest DuP 996 may have therapeutic value in the treatment of diseases which result in cognitive dysfunction.
GLUCOSE FAILED TO REVERSE SCOLAPOLINE-INDUCED DEFICITS IN A WATER-ESCAPE TASK IN RATS. R.D. Holstein* and L.W. Means*. Department of Psychology, East Carolina University, Greenville, NC 27858.

Thirteen Sprague-Dawley male rats were trained to escape in a Pavlovian water maze, where the choice section containing the escape platform was reversed on each trial following a correct choice. Rats were given 1 reference trial/day with the trials separated by intertrial intervals of 1-2 min. Incorrect choices were punished by retaining the rat in the incorrect section for 30 sec. After rats attained a performance level of 92% correct choices/session (CC/S). Injections (s.c.) of 0.5, 1.0 or 2.0 mg/kg scopolamine hydrobromide 30 min prior to testing significantly (p<.01, in each case) reduced the number of CC/S to 6.4, 6.8 and 7.0, respectively, whereas 5 mg/kg methylscopolamine failed to affect CC/S (9.5). In a subsequent experiment using the same rats, no injection, injections with 0.5mg/kg scopolamine alone, or in combination with 100, 250 or 500 mg/kg glucose (i.p.) resulted in 9.6, 6.6, 7.9, 8.5 and 2.2 CC/S, respectively. The rats performed significantly (p<.05 in each case) worse after all injections than when tested after no injection. Thus, the rats were impaired on the task by the CNS activity of scopolamine, and glucose failed to ameliorate the scopolamine-induced impairment.

PARADOXICAL SLEEP IS SELECTIVELY SENSITIVE TO GLUCOSE IN AMNESIC RATS. W.S. Stone and P.E. Gold. Dept. of Psychology, U. of Virginia, Charlottesville, VA 22903.

Poor memory is highly correlated with impaired paradoxical sleep under several conditions. We determined here whether glucose administration, which enhances memory in rodents and humans, also enhances sleep in three amnesic populations: amygdala-kindled, atropine-treated, and old rats. Sleeps of 24 h EEGs were obtained before and after rats received daily kindling stimulation (biphasic square waves, 1 msec, 60 Hz, 250 uA, 1 sec train) for 4-5 weeks or a single injection of atropine (1 mg/kg, IP). EEGs were also obtained from aged (26 mo) Fischer 344 rats which received saline or glucose (100 mg/kg, IP). These rats were then trained on an inhibitory avoidance task (1 min, 1 sec) and later tested for generalization of the learned response.

The results were similar despite different designs and subjects. Glucose selectively increased the duration of paradoxical sleep across 10 min - improved memory - in amnesic but not in respective control populations, including within individual rats in these populations which do not exhibit sleep or memory impairments. These results show that paradoxical sleep measures predict the ability of glucose to improve memory in amnesic and non-amnesic populations, and further, generally, they show that the brain becomes significantly more sensitive to the effects of glucose administration in several amnesic populations. [Supported by DNR (N0001489-J-2165), NIA (AG 07648), and NSF (BNS-901229)].
363.3 EFFECTS OF ANISOMYCIN ON THE LATE CHANGES OF PROTEINS IN PLEURAL SENSORY NEURONS OF APLYSIA PRODUCED BY AN IN VITRO MODEL OF SENSORY INTERNEURONS FROM CEPHALIC TAIL WITHDRAWAL CIRCUIT.

An in vitro analog of sensitization training in Aplysia produces early and late changes in the incorporation of label into specific proteins in pleural sensory neurons. Protein synthesis was blocked by anisomycin (5 μg/ml) 24 h after (late) the training procedure. To determine whether the late changes require translation, we applied the training during the training on the incorporation of labeled amino acids into proteins 24 h after the training.

Experimental pleural-pedal ganglia were exposed to anisomycin (10 μM) for 3 h periods at 1.5 h intervals up to 12 h. After the last exposure, the ganglia were allowed to incubate in control saline for an additional 24 h. Western blotting experiments showed that the amount of several specific proteins that were increased during the training was blocked by anisomycin. These results indicate that at least one of the late changes was due to increased translation of a specific protein. They also emphasize the importance of controlling the possible effects of anisomycin.

363.5 IDENTIFICATION OF PLEURAL NEURONS THAT INHIBIT TAIL SENSORY NEURONS OF APLYSIA: CORRELATION WITH FMRFAMIDE IMMUNOREACTIVITY.

D.L. Cleary and L.H. Byrne.

School of Neurobiology and Anatomy, University of Texas School of Medicine, Houston, TX 77225.

Sensory neurons (SNs) in the pleural ganglia are hyperpolarized by mechanical stimulation of the body outside their receptive fields. FMRFamide is a peptide that has inhibitory effects on SNs, and FMRFamide immunoreactive (IR) neurons are present in the pleural ganglia. Neurons that inhibit the tail SNs have been identified, however.

A cluster of neurons that inhibits inhibitory effects on the SNs. Intracellular stimulation of these cells produced hyperpolarization of SNs. Identified inhibitory cells were injected with Lucifer yellow (LY) and examined with both rabbit and FMRFamide antibodies. Immunoreactive neurons that inhibit the tail SNs have been identified, however.

363.7 SIMULATIONS OF ACTION POTENTIALS, TRANSMITTER RELEASE, AND PLASTICITY OF SENSOMOTOR SYNAPSES IN APLYSIA.

C.A. Cavanagh, D.A. Butler, A. L. Chark, and J.H. Byrne.

Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

A Hodgkin-Huxley type membrane model of an Aplysia sensory neuron has been combined with a model of transmitter release and receive, as well as a model of the synaptic coupling with a follower motor neuron. The membrane dynamics in the model, as well as their modulation by 5-HT, were largely based on voltage-clamp data from the sensory neuron soma. In addition, material balances on Ca2+ and Ca2+ current were considered. Release of transmitter as a cyclic function of Ca2+ current and a linear function of available transmitter. The population of transmitter vesicles was compartmentalized into pools. In order to study its effects on membrane currents, a facilitatory effect of 5-HT on transmitter mobilization was modeled. Parameters of the transmitter mobilization and release model were adjusted by fitting the simulated EPSP to published data recorded during presynaptic voltage-clamp pulses of varying duration (Bachur et al., 1987). The model of synaptic coupling included transmitter accumulation and removal from the cleft, as well as kinetics associated with the transmitter-gated currents in the postsynaptic membrane. Simulations of voltage-clamped sensory neurons produced a good fit to the waveforms of the membrane currents, a facilitatory effect of 5-HT on transmitter mobilization and release, and enhancement of transmitter release. Our goal is to incorporate this model into an in vitro analog of sensitization training in Aplysia in order to simulate the modification of reflex circuits by simple forms of learning.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 17, 1991


An extract of the bag cells inhibited the baseline tail-siphon withdrawal reflex and selectively suppressed long-term sensitization of the reflex (Goldsmith and Byrne, 1990). This suggests that the bag cells may act on the sensory cells to modulate the possibility of synaptic strength of the bag cell-sensory cell synapses. Further experiments were designed to explore the role of the bag cells in the modulation of sensory cell responses. These experiments used an electrophysiological approach to examine the effects of the bag cells on the sensory cell responses to a variety of stimuli, including electrical stimulation of the sensory nerves and the application of chemical substances such as serotonin (5-HT) and FMRFamide. The results suggest that the bag cells may act on the sensory cells to modulate the possibility of synaptic strength of the bag cell-sensory cell synapses.


The effect of 5-HT on synaptic plasticity has been a matter of considerable debate. Some studies have shown that 5-HT enhances synaptic plasticity, while others have shown that it has no effect or even inhibits it. The mechanism by which 5-HT modulates synaptic plasticity is not well understood.

The authors of the paper hypothesized that 5-HT may modulate synaptic plasticity through the activation of specific 5-HT receptors. To test this hypothesis, they used the drug CYP, which is a selective 5-HT antagonist, to block the effects of 5-HT on synaptic plasticity. The results showed that CYP blocks 5-HT-induced spine broadening but not 5-HT-induced anti-accommodation. This indicates that there are at least two distinct 5-HT receptors that mediate these effects.

633.11 SIMPLE NEURAL NETWORK MODELS PROVIDE HEURISTIC TOOLS FOR UNDERSTANDING THE POSSIBLE ROLE OF COMPLEMENTARY NEURON RESPONSES IN APLYSIA. J. Kuglermann, D. Stoddard, and K.R. Weiss. Center for Neurobiology and Behavior, and Dept. of Physiology and Biophysics, Columbia U., School of Medicine and Mt. Sinai School of Medicine, New York, N.Y.

Feeding behavior of Aplysia involves two different stages: a highly stereotyped bite-swallow, and a more diverse orienting response. Bite-swallows are elicited by food stimuli applied to the median region of the lip-tentacles. Orienting turns are elicited by intermediate or far lateral stimulation of the lip-tentacle region, and the magnitude of the response is a function of the distance of the stimulus from the mouth. If a far lateral stimulus is the appropriate orientation response, the orientation is appropriate to the more medial stimulus. No turns are elicited during the execution of a bite-swallow. The above input-output characteristics were modeled by means of a simple neural network consisting of an input layer (sensory neurons) of 5 units (median, left and right intermediate and lateral receptive fields); an output layer (motor neurons) of 3 units (bite, left turn, right turn); and a hidden layer of 5 units. The network was repeatedly presented with 12 combinations of inputs and target outputs, and the connections were adjusted until the network correctly produced the desired output for each of the possible inputs. Different networks were generated by varying the initial conditions and transfer functions for the units. The functional role of individual units was analyzed by "stimulating" and "abating" individual hidden units. The results indicate that simple tests done on different controls can provide misleading information on the actual role of the neuron in generating behavior.


The molluscan neuropeptide FMRFamide has an inhibitory effect on many behaviors, including feeding, locomotion, and escape responses. However, the mechanism by which FMRFamide produces these effects is not well understood.

The authors of the paper hypothesized that FMRFamide may modulate the conductance of siphon motor neurons by acting on voltage-gated ion channels. To test this hypothesis, they recorded the conductance of siphon motor neurons in response to FMRFamide and compared it to the conductance of control neurons. The results showed that FMRFamide produced a significant increase in the conductance of siphon motor neurons, which is consistent with the hypothesis that FMRFamide modulates voltage-gated ion channels.


The authors of the paper hypothesized that long-term changes in synaptic plasticity may be mediated by changes in the morphology of sensory neurons. To test this hypothesis, they recorded the morphology of sensory neurons in response to long-term facilitation and inhibition. The results showed that long-term facilitation produced an increase in the number of sensory neuron varicosities, while long-term inhibition produced a decrease in the number of sensory neuron varicosities. This suggests that long-term changes in synaptic plasticity may be mediated by changes in the morphology of sensory neurons.
633.15 SENSORY NEURON SPIKE BROADENING INDUCED BY TAIL NEURON STIMULATION IN \textit{APLYSIA} IS BLOCKED BY CYPROHEPTADINE. J.P. Perarnau and J.H. Byrne. Dept. of Neurobiology and Anatomy, Uni, Texas Health Science Center, Houston, TX 77025.

Tail shock-induced sensitization in \textit{Aplysia} is known to involve spike broadening in pleural sensory neurons (SNs). This effect on spike duration is mimicked by application of serotonin (5-HT) to the nerve (P1Rs) which receive monosynaptic EPSPs from the C-PR neuron. Sensitization is mediated, at least in part, by 5-HT. We have tested this hypothesis directly by using the 5-HT antagonist cyproheptadine (CYP), which blocks 5-HT-induced spike broadening in SNs (Morgan and Carew, this volume).

Brief pulses of depolarizing current (2ms, 5μA) were elicited singly from pleural sensory neurons. Modulatory input to SNs was activated by stimulation of the pedal nerve P3 (3 trains of 2ms, 5μA pulses at 10Hz). Each train of pulses was duration measured and before and after P3 stimulation. Ganglia from one side of the animal served as controls; those from the opposite side were treated with CYP (200 μM). P3 stimulation significantly increased spike duration in control SNs (k+16%, p<0.05, N=8), whereas SNs treated with CYP showed no significant spike broadening (k+3%, N=4). Thus, CYP blocks spike broadening in pleural SNs by tail nerve stimulation.

Consistent with earlier findings that CYP selectively blocks 5-HT-induced spike broadening without affecting 5-HT-induced changes in excitability (Mercer and Carew, this volume), we have also found that CYP does not affect increases in excitability induced by P3 nerve stimulation. Specifically, in 5 experiments, P3 stimulation in normal sea water produced significant anti-accommodation (k+ increase in spike number=66%, p<0.03) that was unchanged in CYP (k+ increase=60%, p<0.03).

Our results complement previous work by providing direct evidence that 5-HT acts as a neuromodulator in tail SNs of \textit{Aplysia}. Furthermore, our data suggest that endogenous 5-HT acts as different receptor sites in producing different forms of plasticity.

633.17 ANNEXINS ARE EXPRESSED DIFFERENTIALLY IN CNS, INCLUDING SENSORY NEURONS, AND EYE OF \textit{APLYSIA}. I. Cleary, A. Eakin, J.H. Byrne. Dept. of Neurobiology and Anatomy, Uni, Texas Health Science Center, Houston, TX 77025.

In pleural sensory neurons and the eye, 5-HT affects the synthesis of several proteins. One such protein from eye has been partially sequenced and shown to be a member of the annexin family (Raju et al., 90). An interesting property of the annexins is their affinity high binding to membrane phospholipids in the presence of Ca²⁺, suggesting that they could play a role in regulating neuronal function. Using affinity-purified antibodies against the annexin consensus sequence peptide (Kaznel & Dedman, 99), we examined the distribution of annexins in CNS and eye.

acS-immunoreactivity (IR) was selectively, but not exclusively, expressed in sensory neurons in the CNS. In whole mounts, acS-IR appeared to be present in all neurons in the pleural sensory cluster. Larger medial cells were not labeled. In the LE and RE clusters were labeled as well. Only a fraction of neurons in the sensory J cluster of the cerebral ganglion were labeled, and the sensory D cluster of the buccal ganglion was unlabeled. Other labeled cell groups include three medial classes in the cerebral ganglion and a cluster of 5-7 neurons, including B1, on the caudal surface of the buccal ganglia. In the eye, acS-IR was present in the rhabdom, neuropil and optic nerve, but not in the pigment cell layer. The distribution of labeling was clearly different from that of 5HT, which labels only the neuropil or FMRF and myomodulin, which each label a subpopulation of small cells in the photoreceptor layer. Western blots of homogenized pleural ganglia revealed one major cross-reactive protein of ~40 kD, and several minor bands.

The differential expression of annexins within the CNS might be related to the functional role of the IR neurons. One common feature of sensory neurons and the eye is sensitivity to 5-HT. It is conceivable that annexins contribute to the cellular response to modulatory transmitters through alterations of intracellular calcium.


\textit{Aplysia} mechanosensory neurons have a unique tail region that make monosynaptic connections with motor neurons in the pedal ganglia. Although synaptic changes associated with learning and memory have been extensively studied in the sensory-motor synapse, the peripheral mechanosensory terminals have not been characterized. Class III β-tubulin, a tubulin isoform expressed at the onset of neuronal differentiation in vertebrates, is neuronal-specific. Because it also appears to be neurally-specific in \textit{Aplysia}, we have been able to use it here to trace peripheral neuronal structures. The structures were visualized by immunofluorescence, using a monoclonal antibody against class III β-tubulin on frozen cryostat sections from the tail region and from tentacles (tentacles have been described at the EM level: Emery & Audesirk 1977 J Neurobiol: 9:173). Neuronal structures in the tail region were comparable to those of the tentacles' mechanosensory exterior but not to those in its chemosensory groove. A variety of other ultrastructural and neuronal tracing techniques (eg. 2-deoxyglucose, lipophilic dyes, EM) to reveal mechanosensory endings in the tail region are also being explored. Supported by the Canadian NCE and NSERC.

633.19 SENSITIZATION IN JUVENILE \textit{APLYSIA} IS AFFECTED BY DIET. J.W. Flinn, S. Kurtz, C. Honig & West, Dole, S. * George Mason Univ, Fairfax, VA, GPA, Gulf Breeze, FL.

Juvenile \textit{Aplysia} were raised from 50 days post-hatch on algae treated to be low in protein & tryptophan, low in protein, but high in trypt (T), low in trypt, but high in protein (P) and high in both protein & trypt. (W). Animals were tested for sensitization and sensitization (S) or sensitization (S) groups. Sensitization was examined in the ability to escape behavior following tail shock and comparing control (C) and sensitization (S) groups. Partial sensitization analysis showed that the greatest sensitization level was seen in the group at 130 days post-hatch. The sensitization in this group increased over the 3 wk period, reaching 16% at 1255 (S) vs -29 (C). Sensitization in the HI group also increased over the 3 wks and at 130 days was +68% (S) vs -31% (C). The LO & P groups decreased in sensitization over 3 wks and did show sensitization at 130+ days. The data suggests that a trophodynamic mechanism in which the net produced greater sensitization than a diet rich in both. HPLC analyses will be performed to examine the levels of serotonin and other neurotransmitters.
633.21

Two patterns of buccal nerve activity that distinguish ingestion from rejection can be replicated in a reduced preparation in Aplysia. D. W. Merrell and H. J. Chiel. Departments of Neuroscience and Biology, Case Western Reserve University, Cleveland, OH. 44106.

We are studying the neural basis of feeding in Aplysia californica. Our approach has been to associate in vivo patterns of neural activity with specific behaviors and then develop a reduced preparation which produces similar patterns of activity and is suitable for intracellular recording. Using in vivo cuff electrode recordings of buccal nerve 2 (BN2) and the ipsilateral radula nerve (RN) with a simultaneous video record, we have associated two patterns of large unit activity with two types of consummatory feeding behavior. Pattern I is associated with ingestion (biting or swallowing), and is characterized by a burst of large units on the radula occurring together with a burst of large units on BN2. Pattern II is associated with rejection, and is characterized by the RN activity preceding the BN2 activity. The burst of RN activity occurs with retraction in pattern I and protraction in pattern II. To investigate the neural basis of these patterns, we have developed a reduced preparation producing two patterns of large unit activity, termed in vitro patterns, that are qualitatively similar to the in vivo patterns. Using this preparation, we have identified a buccal ganglion neuron that closes the radula when stimulated, appears as a large unit in RN extracellular recordings, and is active during the RN burst in both in vitro patterns. These results suggest that this radula closer motor neuron is active with retraction during ingestion and protraction during rejection, consistent with observations of radula closure during these behaviors. Studying this motor neuron's pre-synaptic cells during both in vitro patterns could provide insights into the neural control of radula closure during ingestion and rejection in Aplysia. Support: BNS-8810757 and ST32G07250.

633.22

Characterization of Pattern Triggering Neurons from Aplysia's Buccal Ganglion In situ and in Culture. H. J. Chiel 1,2 and Jigang You 1. Departments of Biology and Neuroscience, Case Western Reserve University, Cleveland, OH. 44106.

Pattern triggering neurons have been described in the buccal ganglion of Aplysia (Susswein and Byrne, J. Neurosci. 8:204-8, 1988). Do these neurons induce bursting due to their intrinsic properties or by their connections to other neurons? To answer this question, we have studied these neurons in the buccal ganglion and in isolated cell culture. Cells were identified using the criteria of Susswein and Byrne. The only neurons which we found that triggered patterns also generated action potentials, and thus might correspond to B31.35 or 37. In the intact ganglion, cells were injected with Lucifer yellow and their morphology was visualized. Of 8 cells filled in 8 different ganglia, 5 showed a single axon which crossed the midline of the ganglion via the buccal commissure; the remaining three had axons projecting via peripheral nerves (buccal nerves 2, 3, or the cerebral buccal connective). In the intact ganglion, low calcium solutions blocked the ability of the neurons to trigger patterns, but caused spontaneous bursts to occur in B4. High divalent cation solutions greatly reduced the ability of the neurons to trigger patterns, but fast IPSPs and slow EPSPs from these neurons to B4 were clearly observed. Maps of ganglia were drawn with a camera lucida; pattern triggering cells were identified physiologically, and cells were removed for isolated cell culture. In 10 experiments (one in which the cell was cultured in isolation, and 9 in which it was co-cultured with B4), pattern triggering neurons showed irregular firing in response to large depolarizing currents in vitro, but did not burst. These results support the hypothesis that the pattern-triggering neurons induce bursting in the buccal ganglion by means of synaptic connections to other neurons. Supported by NSF grant BNS-8810757.

633.23

633.24


The nudibranch, Melibe leonina, feeds by rhythmically sieving small crustaceans from the water with its large oral hood. Previous studies demonstrated that the frequency of feeding movements is proportional to the concentration of prey. The purpose of this study was to determine what qualities of prey are important in modulating the frequency of Melibe feeding. During a typical experiment 4 animals were placed in an aquarium and exposed to one of the following treatments: 1) Artemia at a concentration of 1500/ml; 2) Artemia-conditioned seawater; 3) small (350 μm) Sephadex beads soaked in Artemia-conditioned water. The feeding frequency of each animal was then monitored for 20 min. Both conditioned water (olfactory stimulus) and Sephadex beads (tactile stimuli) caused appetitive behavior (orientation of the oral hood) and a significant increase in the rate of feeding. When both were added together, they produced a larger increase than either did alone, but still not as great a response as the intact Artemia. Thus, we conclude that Melibe detects the presence of prey using at least three different signals: smell, touch, and perhaps movement of live prey. This study was supported by the U.N.H. BRSG program.

OPTICAL MEASUREMENT OF ACTIVITY IN THE MELIBE LEONINA Buccal Ganglion. Larry B. Cohen, Win Watson, Jim Tribranch, Chen X. Park and Jang-Young Ku. Dep. of Physiology, Yale University School of Medicine; Dep. of Zoology, University of New Hampshire; and Marine Biological Laboratory. Molluscan buccal ganglia have been used to study the neural basis of feeding behavior. However, detailed information about the underlying neural circuits is lacking for most buccal preparations because of their complexity. The buccal ganglion of Melibe leonina, with only 40-50 neurons, is relatively simple and transparent. Optical recordings from this ganglion might be able to monitor all the action potentials in all of the neurons. Using ganglia stained with a merocyanine (JPM1124) or an oxonol (RH155) dye we tested the completeness of the recording. In one preparation we were able to detect activity in more than 90% of the cells. In a recording made during rhythmical output from the ganglion we detected activity in about 50% of the neurons. However, in many preparations the lighting led to a photo-stimulation. We plan to try reduced dye concentration and additional dyes to avoid this effect. Supported by NIH grant number NS08437 and a UNH Summer Faculty Scholarship.

633.25


Two types of acquisition responses have been observed in Clione. (1) Buccal cone eversion can be triggered by direct contact with prey. The threshold for such a "fast strike" is very high, and an unsuccessful strike results in immediate withdrawal of the buccal cones. (2) Perfusion of prey extract across the head such a "fast strike" is very high, and an unsuccessful strike results in immediate withdrawal of the buccal cones. A neurons involved in prey acquisition. One group (A neurons) initiates lip retraction, and another group (B neurons) then closes the lips (C neurons). Serotonin activates A neurons and thus favors buccal cone eversion. Dopamine favors buccal cone eversion. Dopamine depolarizes both A and B neurons, but the effect is much more powerful in B neurons. Dopamine thus favors buccal cone retraction. Superfusion of GABA produces simultaneous inhibition of B neurons and excitation of A neurons. The difference between a fast strike and hunting behavior may hinge, at least in part, on the interplay between several modulators/transmitters which regulate the excitability of feeding neurons.
634.1 DEPLETION OF SOMATOSTATIN BY CYSTEINE SELECTIVELY INHIBITS DARK-ONSET FEEDING IN RATS. D. Feifel and D.J. Vazquez. Department of Psychology, University of Toronto, Toronto, Canada M5S 1A1.

In a previous study we demonstrated that blockade of endogenous GRF by injection of GRF antisera into rat hypothalamus, selectively inhibited dark-onset feeding, but not feeding at other circadian time points. This suggested that endogenous GRF plays a role in the circadian organisation of feeding. We have also reported that intrahypothalamic somatostatin activity is an important output for GRF-induced feeding. This study sought to determine if endogenous somatostatin, like GRF, also plays a role in circadian-specific feeding.

Male Wistar rats were housed under a 12/12 light-dark photoschedule and treated for their feeding response to subcutaneous injections of cytochrome (300 mg/kg) (known to deplete body somatostatin). Results indicated that cytochrome injections selectively inhibited food intake (5%) at dark-onset but not at other time point tested.

These findings suggest that somatostatin, like GRF, is involved in the mechanism which organizes the circadian pattern of feeding in rats. This work was supported by NSERC grant 35036 to F.J.V. D.P.F. was supported by a NRC Postgraduate Scholarship.

634.2 THE EFFECT OF Nle<sup>6</sup>,D-Phe<sup>7</sup>-[α-MSH] AND ITS FRAGMENTS ON CORE TEMPERATURE IN RATS. L.H. Rabiee* and M. Camacho-Cholet. Kalamezo College, Kalamazoo MI 49001, The Upjohn Company, Kalamazoo, MI 49001.

The research of Lipton and coworkers indicates that α-melanocyte stimulating hormone (MSH) exerts antipyretic and hypothalamic actions in the rabbit. Research on MSH and temperature in the rat has utilized peripheral or ventricular injections of MSH fragments and has yielded inconclusive findings. More direct application of MSH or an analog of MSH might produce more conclusive results. The present experiments examined the effects of a potent MSH analog (Nle<sup>6</sup>,D-Phe<sup>7</sup>-[α-MSH]; NDF-MSH) and its fragments (NF-MSH<sub>7</sub>, NDF-MSH<sub>6-13</sub>, NDF-MSH<sub>1-13</sub>) all provided by Dr. Tomi Sawyer, The Upjohn Company) on temperature when injected into the anterior hypothalamic/medial preoptic area (AV/PoA). Results indicated that rats receiving 5 pmol of NDF-MSH/5 μl NAcCl displayed significantly greater temperatures than those receiving the vehicle. (F(5,10)= 5.00, p<0.02). The least amount of variance was observed in those subjects receiving 5 pmol NDF-MSH. The second experiment indicated that AV/PoA injections of 5 pmol/5 μl NDF-MSH and its 4-10 and 7-13 fragments significantly elevated temperature while injections of vehicle and of the 1-7 fragment did not, (F(4,52)=1.72, p>0.05), a finding consistent with other assays. Studies designed to eliminate the possibility of a pyrogenic contaminant in the effective solutions are currently underway.

634.3 CENTRALLY INJECTED BOMBESIN ELICITS GROOMING IN DEVELOPING RAT PUPS. R. Flugherm and R.M. Martin, 1,2, Psychology & Pharmacology, University of Ottawa, Ottawa, Canada, K1R 6B5.

In adult rats (i.c.v.) injection of the tetradecapeptide bombesin (BB) elicits a scratching response and consequent face washing. In this study, aimed: (1) to ascertain if i.c.v. BB would elicit grooming behaviour in developing rats and (2) to characterize the ontogeny of circadian forcing of this response. Briefly, anesthetized pups of 1-20 days of age were injected i.c.v. with BB (0.01-1.0 μg/kg) or saline (n=7/dose/age) and the videotaped responses scored for grooming and face washing. In this study, compared to saline, which was least sensitive to i.c.v. BB, there were dosage-related grooming responses in which no NaCl control was significantly different from that of the saline controls. These results indicate that BN binding sites in developing rats and (2) to characterize the ontogeny of circadian forcing of this response. Briefly, anesthetized pups of 1-20 days of age were injected i.c.v. with BN (0.01-1.0 μg/kg) or saline (n=7/dose/age) and the videotaped responses scored for grooming and face washing. In the 1-7 age group, compared to saline, which was least sensitive to i.c.v. BB, there were dosage-related grooming responses in which no NaCl control was significantly different from that of the saline controls. These results indicate that BN binding sites in developing rats are particularly distinguishable at postnatal day 20.

634.5 HIF-1 ALTERS PAVLOVIAN CARDIOVASCULAR CONDITIONING, CONCOMITANT CORTICAL NEURAL ACTIVITY, & DELAYS EXTINCTION IN RABBITS Linda L. Hernández, Philip J. Tollison* & Charles M. Gibbs, Dept. VA Hospital & U. South Carolina, Columbia, SC 29201.

The effect of repeated administration of ceruletide (100 μg/kg/day, 1P, 3days) on mouth movement and SCH 23980 binding to striatal membrane was examined in chronic Pavlovian-Fleming (nicotine) extinguished (FP2) treated rats (25ng/kg, IM, every 3 weeksx10) and saline treated (control) rats. SVMs after final nicotine injections showed decreases in mouth movements (teeth chattering, chewing, licking, opening of mouth, perioral tremor) and an increase in SCH 23980 binding to striatal membrane in the FP2-treated rats. High amplitude EMG discharge (4c/s), which correlated to perioral tremor was recorded from the masseter in the FP2-treated rats. Repeated ceruletide injection suppressed mouth movements and normalized SCH 23980 binding to striatal membrane in the FP2-treated rats. The effects of ceruletide on mouth movement and D1 receptor continued for 6 days after final ceruletide injection. These findings indicate that systemically administered ceruletide affects the D1 receptor and that an increase in D1 receptor function may play an important role in the pathogenesis of tardive dyskinesia.


A previous study in our laboratory has shown that prenatal administration of ACTH-1-24 or nicotine decreases the lordosis response in female rats tested as virgins (Alves and Strand, 1990; See for Neuropeptides, 1991). This study was conducted to investigate whether postnatal treatment of these substances would affect the reproductive capacity of female rats. Sprague-Dawley rat pups were injected s.c. with either ACTH-1-24 (0.1mg/kg), nicotine hydrochloride tartrate (0.2mg/kg) or saline vehicle once daily from postnatal day one (day of birth) to day 27. At 4 weeks of age females were checked for vaginal opening as a sign of sexual maturation. At approximately 60 days of age, these animals were tested for sexual behavior as virgins. The sexual maturation of ACTH treated animals was significantly delayed when compared to nicotine treated and control animals (p<0.001). Animals from both groups displayed decreased female sexual behavior, having significantly lower lordosis quotients and lordotic quality scores compared to control animals (p<0.001). Hypothalamic 5-HT (rat) fiber densities were significantly increased by 5-HT uptake was significantly increased in ACTH treated animals (p<0.02) but unchanged in nicotine treated animals compared to controls. This increase in hypothalamic 5-HT in ACTH treated animals could account for the decrease in female sexual behavior seen in these animals. We hypothesized that sexual differentiation of the brain in the female rat is susceptible to postnatal manipulation with ACTH or nicotine. We are currently investigating plasma sex steroid levels and immunohistochemistry for the neurotransmitters involved in female sexual behavior. This study was supported by the Council for Tobacco Research.

634.8 EFFECTS OF MESENCEPHALIC INJECTIONS OF NEUTROTENSIN-13 AND NEUTROTENSIN-33 ON CENTRAL GRAY SELF-STIMULATION. P.P. Rompré1 and A. Gratton2. 1 CSBN, Psychology Dept., Concordia University, Montreal, Quebec, H3G 1M8 and 2 Douglas Hosp. Res. Ctr., McGill University, Verdun, Quebec, H3H 1P5.

634.9 EVIDENCE FOR AN INTERACTION OF NEUTROTENSIN WITH THE A-1 SUBTYPE OF ADENOSINE RECEPTORS. F. J. Jolicoeur and D. Bélair. Sherbrooke, Sherbrooke QC. Canada J1H 5N4. We have reported that pre-administration of the mixed adenosine antagonist, theophylline, systemically abolishes the hypothermic and hypokinetic effects of neurtensin (Soc. Neurosci. Abstr. 1990). In order to better delineate this interaction, we examined the effects of pre-treatment with pharmacologic drugs purported to act specifically at the A1 or A2 receptor subtype, either as agonists or antagonists. For each drug examined, a dose was chosen which, when given alone, was injected SC 15 min prior to the intracerebro-ventricular administration of several doses of neurtensin (0.05 - 50.0 µg). L71 (A1 agonist) significantly increased both the hypothermic and hypokinetic effects of neurtensin. On the other hand, IBMX (A1 agonist) significantly decreased the hypothermia but not the hypokinetic properties of neurtensin. Prior administration of CCP or DMPP (A2 agonist and antagonist respectively) did not alter these effects of neurtensin. These results suggest that neurtensin induces hypothermia and possibly hypokinesia might be mediated via an interaction with adenosine A1 receptors. However, an interaction of the peptide with phosphodiesterase might also be of importance, since theophylline and IBMX are well known inhibitors of this enzyme.

634.10 THE EFFECTS OF INTRACEREBRAL INJECTIONS OF TWO NOVEL NEUROKINS (NEUROKININ A AND NEUROKININ K) ON THE EXPRESSION OF MALE RAT SEXUAL BEHAVIOR. Peter Malen, Kevin Shortt2, and Wayne Dumas. Dept. of Psychology, Illinois State University, Bloomington, IL 61701.

634.11 CHRONIC ADMINISTRATION OF ANGIOTENSIN II AND DuP 753 INCREASES GROWTH OF EMBRYONIC RAT HEART CULTURED IN OCULO. R. Hunt, A. Torres, and D. C. Tucker. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

634.12 INHIBITION OF CALCITONIN-INDUCED ANOREXIA BY ANTIADRENERGIC DRUGS. P. de Beaucoudray. Laboratoire de Pharmacologie, CHU Côte de Nacre, 14032, Caen, FRANCE.

Calcitonin is a peptide hormone secreted by the thyroid gland, which acts primarily on calcium homeostasis. When injected peripherally or centrally, calcitonin reduces food intake. The effects of chronic antiserotonin treatment on calcitonin-induced anorexia were tested in rats.

Daily intraperitoneal injections of 9 different antiserotoninns, two anxiolytics, and one neuroleptic were made during three weeks, and the daily food intake of the animals were recorded every day during the last week. The drugs were tested in 2 groups of 8 animals, and for each drug a control group of 8 animals was daily injected with saline. On day 21, the animals received an intraperitoneal injection of 40 units of calcitonin.

The results show that calcitonin reduces food intake by more than 90% in all control animals and animal treated by the anxiolytics (meprobamat, 100 mg/kg, clonazepam, 60 mg/kg), and the neuroleptic (chlorpromazine, 10 mg/kg), but not in animals treated with the antidepressant drugs. The most effective drugs were clomipramine (15 and 25 mg/kg), imipramine (25 mg/kg), amitriptyline (25 mg/kg), trimipramine (30 mg/kg), and maprotiline (25 mg/kg). Conversely, flunitrazepam (15 mg/kg), flurazepam (30 mg/kg), xilazine (30 mg/kg), and trazodone (100 mg/kg) inhibited the effects of calcitonin in some animals, but the results were not significant.

These results demonstrate that tricyclic antidepressants can have a specific inhibitory effect on a behavior induced by a centrally active peptide.
634.13

SELECTIVE ANTAGONISTIC PROPERTIES OF THE C-TERMINAL FRAGMENT OF CALCITONIN GENE-RELATED PEPTIDE, H-CGRP$_{9-37}$

D. Ménard, 1A. Fournier and F. Jolicoeur. Depts of Psychiatry and Pharmacology, Univ. Sherbrooke, Sherbrooke QC, Canada J1H5N4 and 1NRS-Santé, Pointe Claire, Qc, Canada.

Results from our recent studies indicated that a C-terminal fragment of the peptide, CGRP$_{9-37}$ could block certain in vivo and in vitro effects of h-CGRP (JPET 254:123, 1990). In order to further characterize the antagonistic properties of this fragment, its activity against the following h-CGRP nerve endings was examined: hypokinesia, anorexia, analgesia, hyperthermia and catalepsy. These effects were observed following intracerebroventricular (ICV) administration of 20 μg of the peptide. First, CGRP$_{9-37}$ alone injected ICV in several doses (10-80 μg) did not alter any neurobehavioral measurements. However, prior administration of the fragment at 40 μg significantly attenuated and at 80 μg systematically abolished the anorexia and analgesia produced by CGRP. The other neurobehavioral effects of CGRP were not affected. These results support the existence of heterogenous receptors mediating central effects of CGRP.

Follow-up structure activity studies, using various C-terminal fragments of the peptide, indicated that CGRP$_{9-37}$ was the minimal length required to obtain antagonistic activity.

634.15

DIFFERENTIAL EXPRESSION OF NEUROPEPTIDE mRNA AS RELATED TO AGE IN APLYSIA.


With increased age the Aplysia nervous system manifests behavioral, physiological and morphological changes (Perez et al., 1984, Peretz, 1989). The basis of these age related changes is unknown. Although environmental factors may influence the effects of age on the Aplysia nervous system (Zolman & Peretz, 1987), as yet the role of genetic factors has not been examined. Here we describe age-related differences in the mRNA expression of Phe-Met-Arg-Phe-NH$_2$ (FMRFamide) and egg-laying hormone (ELH) in the abdominal ganglion, a part of the Aplysia CNS. Northern blot analysis of RNA isolated in ganglia from young (ca 80 days old), mature (ca 140 days old) and aged (over 240 days old) animals revealed two mRNA species of 1.4 and 3.2 kb for FMRFamide mRNA and a single mRNA species of 0.8 kb for ELH. In young animals FMRFamide mRNA was exhibited at low levels, with a 1.5 fold increase in the mature group and a 3-3 fold decrease in old animals. In contrast, the ELH mRNA levels increased 2 fold from young to mature animals, but in old animals the increase was ca 25 fold. These studies show that age has a differential effect on mRNA of these two peptides in the abdominal ganglion, thus the differential effect of age on abdominal ganglion neurons reported earlier (Perez et al., 1984) may well have a genetic basis. Our findings suggest that there is a genetic basis for the age-related change in behaviors mediated by ELH and FMRFamide in Aplysia.

634.16

CONOPRESSIN G SUPPRESSION OF THE SEROTONIN-SENSITIVE K$^+$ CURRENT IN APLYSIA SENSORY NEURONS.

M. Martinez-Padron, L. Edstrom and K. Lukowiak. Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N 4N1 Canada.

Single-electrode voltage clamp techniques were used to analyze the mechanisms of action of the excitability and action potential shape changes caused by conopressin G on Aplysia siphon sensory neurons (Lukowiak, 1989). Bath application of micromolar concentration of conopressin G reversibly decreases the total outward membrane current produced by depolarizing voltage commands to -20 mV. The threshold for this action is in the 10$^{-6}$ M range. The reversal potential of the outward current if shifted in the positive direction by about 50 mV per 10-fold decrease in the extracellular concentration of potassium. Changes in the extracellular concentration of sodium also little or no effect on the reversal potential. Intracellular injection of CaCl$_2$ blocks the outward potassium current by about 15% and abolishes or greatly reduces the effects of conopressin G, suggesting that the peptide is acting to reduce the outward K$^+$ current. The current is highly resistant to tetrodotoxin, 4-aminopyridine. Taken together, these data suggest that conopressin G decreases the 5-like current in abdominal siphon sensory neurons.

635.1

FETAL ETHANOL EXPOSURE ALTERS GLUCOCORTICOID AND B-ENDORPHIN RESPONSES TO STRESS.

J. Weinberg and C. Jolicoeur. Depts of Psychiatry and Pharmacology, Univ. Sherbrooke, Sherbrooke QC, Canada J1H5N4 and 1NRS-Santé, Pointe Claire, Qc, Canada.

The present study examined effects of fetal ethanol exposure on responses of the hypothalamo-pituitary-adrenal (HPA) axis to stress. FETAL ETHANOL EXPOSURE ALTERS GLUCOCORTICOID AND B-ENDORPHIN RESPONSES TO STRESS. J. Weinberg and C. Jolicoeur. Depts of Psychiatry and Pharmacology, Univ. Sherbrooke, Sherbrooke QC, Canada J1H5N4 and 1NRS-Santé, Pointe Claire, Qc, Canada.

β-ENDORPHIN RESPONSES TO STRESS. J. Weinberg and C. Jolicoeur. Depts of Psychiatry and Pharmacology, Univ. Sherbrooke, Sherbrooke QC, Canada J1H5N4 and 1NRS-Santé, Pointe Claire, Qc, Canada.

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635.3 EFFECTS OF PRENATAL ALCOHOL EXPOSURE AND ACOUSTIC TRAUMA ON THE RAT'S AUDITORY BRAINSTEM RESPONSE (ABR) IN PREGNANT RATS: M. J. Renner1, A. J. Bennett*1, D. R. Widman*2 & B. A. Blanchard*3 1 Department of Psychology, Memphis State U, Memphis, TN 38152 2 Department of Psychology, State U of New York, Albany, NY 12222 3 Department of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208 Prenatal exposure to alcohol is associated with deficits in learning, attention, and motor performance. This study was conducted to identify the characteristics of spontaneous behavior that may contribute to these deficits. Pregnant female rats were fed one of three diets from gestation day 6-20: a liquid diet in which 35% of calories were derived from ethanol (35%, n=6 pups); liquid diet without ethanol, fortified with mevinol for equivalent caloric intake to the 35% ETOH diet (0%, n=8 pups); and standard lab chow (LC, n=7 pups). After 200, motor and pups were given standard lab chow. At 80 days of age pups were videotaped during 3 consecutive nights' access to a trapezoidal arena containing two objects. Tapes were scored for exploration, general activity, grooming, and object interactions, using a standard protocol (Remer & Selzner, J Comparative Psychol, 1991, in press). This method minimizes subject stress and maximizes expression of the subjects' behavioral repertoire. Objects with different stimulus characteristics were treated differently, consistent with other studies using these techniques. No effect of prenatal maternal diet was found for any molar-level measure of behavior, including interactions with objects. Micro-analysis of behavior sequences used during object interaction, however, revealed increases in behavioral complexity over days for LC group and decreases for the 35% group; the 0% showed different effects for manipulable (decreases) and non-manipulable (increases) objects. These results are consistent with the hypothesis that prenatal exposure to ethanol exerts its effects on information-processing capacity through subtle behavioral and attentional changes.

635.4 SYNERGISTIC EFFECTS OF SHORT-TERM MATERNAL EXPOSURE TO ETHANOL AND EARLY SENSORY DEPRIVATION ON VISUAL DISCRIMINATION LEARNING IN RATS. M. L. LeBlanc, M. L. Schreiber and A. G. Balaban. Mount Sinai School of Medicine, New York, New York 10029 Early sensory deprivation during cerebral cortical development has been shown to produce cortical thinning and disorganization, but not necessarily a detectable functional deficit. Nevertheless, the animal may have been rendered vulnerable to other adverse influences. Possible synergistic effects of ethanol exposure and light deprivation during development were tested. Offspring of Long-Evans rats who had received a total oral dose of 10g/kg of ethanol on gestation days 14 and 15 (E) and lab chow controls (C) were exposed to a reduced (50%) or a full (100%) light regimen from the first day to 45. They were then trained in a shock-motivated two-choice box to discriminate between horizontal and vertical alternating black-and-white stripes.

The ED rats were impaired in learning the discrimination: they needed more trials than the CD rats to reach criterion. In contrast, the ED group did not differ from the CL rats. The results thus indicate that acute maternal consumption of ethanol and adverse early postnatal rearing environment can synergistically produce functional deficit.

635.5 DEVELOPMENTAL REGULATION OF GABA EXPRESSION IN THE SUPERIOR COLICULUS OF SWISS WEBSTER MICE IS AFFECTED BY PRENATAL ALCOHOL EXPOSURE. C.S. Zajac and D.L. McGunagle*. Fetal Alcohol Res Ctr, Wayne State Univ., Detroit, MI 48201. This study was undertaken to determine the developmental regulation of glutamic acid decarboxylase (GAD) expression in the superior colliculus (SC) of Swiss Webster mice and the effect of prenatal alcohol exposure (35%) on this regulation. To that effect, newborn (NB) and gestation day (GD) 15 fetal mouse pups were sacrificed and fixed for GAD immunohistochemistry, lyophilized, and stored at -70°C. GABA levels were determined for lyophilized SC using the "GABAcube" method. The SC of NB male pups prenatally exposed to alcohol had significantly greater levels of GABA than all other groups tested including NB females with the same treatment. There were no significant differences between other groups.

The superior colliculus plays a significant role in coordinating reflex responses to visual, auditory, vestibular and somesthetic input. The significantly higher GABA levels in newborn male pups (but not in females) confirm other studies showing a sex related difference in the response of neurons to prenatal alcohol exposure.

635.6 SPONTANEOUS BEHAVIORAL ORGANIZATION IN RATS PRENATALLY EXPOSED TO ETHANOL. M. J. Renner1, A. J. Bennett*1, D. R. Widman*2 & B. A. Blanchard*3 1 Department of Psychology, Memphis State U, Memphis, TN 38152 2 Department of Psychology, State U of New York, Albany, NY 12222 3 Department of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208 Ethanol exposure during cerebral cortical development significantly alters the processing capability through subtle behavioral and attentional changes.

635.8 BRAIN CATACHOLAMINE CONTENT DURING RECOVERY FROM STRESS IN FETAL ETHANOL-EXPOSED RATS. P.K. Budenz and J. Veleg, Dept of Anatomy & Neurobiology, Univ of Missouri, Columbia, MO 65212; Dept of Anatomy, Univ of British Columbia, Vancouver, BC, V6T 1Z3 Effects of fetal ethanol exposure on brain catecholamine (CA) content during recovery from restraint stress were examined. Sprague-Dawley male and female rats from fetal ethanol-exposed (PEE), pair-fed (PF) and ad lib计提-fed (C) conditions were tested prior to stress (basal), immediately after 60 min restraint, or following 15, 30 or 60 min recovery. Tissue CA were measured in hippocampus (HIP), hypothalamus (HY), and cortex (CX) by HPLC-EC. Results resulted in a reduction of HY norepinephrine (NE) content that persisted for at least 30 min following stress termination. NE levels returned to basal levels by 60 min of recovery. A similar response was seen in CX NE but the decrease was smaller and longer lasting. Cortical NE was reduced overall in PEE and PF compared to C, animals, whereas as HY NE was reduced in PEE females but not males. There was a sex difference in HIP NE but no sex or treatment effects in epinephrine (E) or dopamine (DA) content of HY or CX. Reduction of neuronal NE content after restraint that continues throughout 30 min of the recovery period suggests that the effects of stress continue following the cessation of the stressor. The differential response seen in PEE animals may be related to the pituitary-adrenal hyperresponsiveness previously observed. (Supported by NIAAA grants AA05893 & AA0107 (PKR) & AA07789 (JV).)
635.9 EVIDENCE THAT MODERATE ETHANOL CONSUMPTION DURING GESTATION DOES NOT ALTER THE METABOLOTROPIC OR IONOTROPIC COMPONENTS OF HIPPOCAMPAL GLUTAMATE RECEPTORS AT OFFSPRING 21-DAY-OLD. A.K. Shetty*, S. R. Lopez*, C. F. Sanchez, L. L. Paxton* and D.D. Savage. Department of Pharmacology, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

Prenatal exposure to a relatively high maternal blood concentration (BEC) decreases quisqualate metabolotropic stimulated inositol-1-phosphate (IP1) accumulation in the hippocampal formation (HPF) and brainstem serotonergic neurons. Using high performance liquid chromatography (HPLC) we have estimated the concentration of IP1 in 21-day-old offspring during early development between the two groups. Ethanol superfused over the surface elicited two different responses in both groups; low dose excitations and high dose inhibitions. However, monoaminergic neurons in the a decreased sensitivity to locally applied ethanol. The dose-response curve was significantly shifted to the right in this group. These results suggest that ethanol during early development disturbances the normal structure of brainstem serotonergic neurons, as well as their response to acute ethanol. Supported by Swedish MRC, grant G 6560.


Fetal alcohol syndrome (FAS) is one of the highest known causes of mental retardation in the western world. Previous research has shown behavioral and electrophysiological abnormalities in human infants bom to mothers who consumed alcohol during pregnancy. A rat model which has several qualitative similarities to the human condition, has recently been developed. In the present study this rat FAS model was used to evaluate the effects of prenatal alcohol exposure. The sample size was 9 rats in 3 groups: control, alcohol, and alcohol plus diet groups. Alcohol was administered at 1% and 2% in the diet from postnatal day 13 to postnatal day 15. The results suggest that maternal behavior in 35% EDC dams should be considered in neonatal state regulation studies that use this animal model. (Supported by NIAAA #AA0605).


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635.12 EFFECTS ON IN UTERO ETHANOL EXPOSURE ON THE POSTNATAL DEVELOPMENT OF BERGMANN GLIA IN RAT CEREBELLUM. A.K. Shetty* and D. E. Phillips. Dept. of Biology, Montana State University, Bozeman, MT 59717.

Animals prenatally exposed to ethanol exhibit a delay in the postnatal migration of cerebellar neurons from the external granular layer to the internal granular layer. Since these neurons use the long processes of Bergmann glial cells across the molecular layer as a guide during migration, we have examined the effects of prenatal alcohol exposure on the postnatal migration of Bergmann glial cells. Using autoradiography, we have determined that ethanol exposure delayed the migration of Bergmann glial cells. These results are consistent with previous studies demonstrating that prenatal alcohol exposure retards the postnatal development of Bergmann glial cells in the cerebellum which may contribute to the delayed migration of granule cells. Supported by NIAAA AA06484 and RR08119.

635.13 THE EFFECTS OF PRENATAL OR POSTNATAL ETHANOL EXPOSURE ON SPATIAL LEARNING IN RATS. M. L. Bangoura. Florida Atlantic Univ., Boca Raton, FL 33431.

The purpose of this experiment was to investigate the effects of pre- and postnatal ethanol (E0) exposure on spatial learning in 30 & 60 day old Sprague-Dawley rats. (Charles River). Pregnant rats were randomly assigned to one of 3 groups: liquid diet (Biosev) containing 35% ethanol (E0), liquid diet (Biosev) containing 35% ethanol (E0) or ad lib diet (Purina). The postnatal exposure group self administered either a 15% sweetened (Nutrasweet) E0 solution or tap water from postnatal day 13 to 17. Spatial learning performance was measured over 5 consecutive days beginning on either day 30 or 60, in a Morris swim maze. There was no significant difference in performance between the pre- and postnatal exposure groups. At both ages, the postnatally exposed group showed greater deficits in learning. These data suggest that prenatal E0 exposure produces greater deficits in spatial learning than prenatally E0 exposure.
LACK OF RECOVERY FROM MEMORIAL AND NEUROBIOLOGICAL DEFICITS FROM HIGH-PEAK BIC FROM RESULTING FROM EARLY POSTNATAL EXPOSURE TO ETHANOL. P.L. Green, J.A. Eraz-Granados & A. Amzel, Department of Psychology and Institute for Neuroscience, University of Texas, Austin, Texas, 198712

Electrolytic hippocampal lesions at 10-11 days of age (P10-11) (Lobeaugh et al., 1989), postnatal hippocampal exposure to x-irradiation (Graz-Granados et al., submitted) and postnatal exposure to ethanol from P4-10 that results in high daily peak blood ethanol concentration (BEC) (Greene et al., submitted) significantly retard the acquisition of patterned (single) alternation (PAL), a form of memory-based learning, with 60-s but not 30-s intertrial interval (ITI) in rat pups tested on P17-18. We examined the effect of early postnatal (P4-10) exposure to E10H with high- (H) and low- (L) peak BEC. Artificially-reared pups in the L condition were fed a 10.2% E10H-adulterated diet (containing an isocoric control diet) on 4 consecutive hourly feeding days each day and undulaterated diet on the remaining 20 feedings. L pups received 1.7% E10H-adulterated diet on all 24 days. H pups at P60-86 were impaired in PAL learning relative to L and control pups at 60-ITI, the same ITI at which PA deficits were observed in infant rats. Group H PA weights were significantly reduced, relative to Group-L and control weights, at 86 days, mirroring the result at 21 days of age. These results suggest incomplete recovery from the membrane and neurobiological teratological effects of the high-peak ethanol exposure regimen in infancy. Supported by NIAAA grant AA07552.

DRUGS OF ABUSE—CELLULAR EFFECTS OF ETHANOL

MARLED INHIBITION OF DOPAMINE RELEASE: A COMMON NEUROBIOLOGICAL SUBSTRATE FOR ABSTINENCE FROM DRUGS OF ABUSE. Z.L. Rossetti, F. Meli, S. Carboni and G.L. Ciness, Dept of Neuroscience, Univ. of Cagliari, Italy.

Behavioral and biochemical evidence indicates that the dopa­mine (DA) pathways mediating reward and pleasure are deeply in­volved in dependence from drugs of abuse. By using the microdialysis technique, we studied the changes in DA efflux from the me­dial septum system in rats by phenobarbital treatment with mor­phine, alcohol, or cocaine, and after withdrawal from these drugs. Ethanol. In ethanol-dependent rats (5 g/kg p.o. every 6 h for 6 days) withdrawal symptomatology was associated with a progressive decrease of DA extracellular levels. DA reached 20% of controls when abstinence symptomatology was fully established. Ethanol (5 g/kg p.o.) suddenly reversed both abstinence symptomatology and inhibition of DA outflow. Cocaine. In chronic cocaine-treated rats (15 mg/kg twice per day for 18 days) extracellular DA concentra­tions were decreased to 35% of controls for at least 5 days after the suspension of the treatment. Cocaine (10 mg/kg i.p.) immediately reversed this effect. Morphine. In morphine-dependent rats, (s.c. 70­mg morphine pellets for 5 days) the gradual onset of abstinence symptomatology following by pellet removal was paralleled by a progressive increase of DA levels. DA efflux reached a minimum (23% of controls) when opiate withdrawal was clearly manifested. Morphine (100 mg/kg s.c.) suddenly reversed both abstinence symptomatology and inhibition of DA release. Thus, DA system mediating the hedonic and reinforcing actions of drugs of abuse is impaired in drug dependence. The inhibition of DA transmission in the mesolimbic system appears to be a common neurobiological substrate for abstinence from drugs of abuse.

INFLUENCE OF ETHANOL ON NEUROBLASTOMA AND GLIOMA CELLS IN VITRO: GLYCOSPHINGOLIPIDS. VITRO: GLYCOSPHINGOLIPIDS. INFLUENCE OF ETHANOL ON NEUROBLASTOMA AND GLIOMA CELLS IN VITRO: GLYCOSPHINGOLIPIDS.


Chronic (7day) ethanol (E) exposure has been reported to selectively inhibit bradykinin (BK)-stimulated [3H]inositol phosphate (IP) production in N1E-115 cells, while having no effect on this response to neu­rokinin (NF). In order to determine whether chronic E also produces selective effects on receptor-mediated increases in intracellular free calcium, [Ca2+]i, N1E-115 cells were exposed to 1000 nM E for resting [Ca2+]i were also determined. Confluent cells grown in DMEM with 10% fetal calf serum were incubated in DMEM containing 80 mM HEPES buffered salt solution and [Ca2+]i determined fluorometrically. Chronic E reduced by 43% the [Ca2+]i response to BK, but had no effect on the same response to NF. Resting [Ca2+]i values (nM) for control and chronic E treated cells were 65±12±50±80, respectively. Acute E (3-4 min), as high as 400 mM had no significant effects on resting [Ca2+]i. It is concluded that BK-mediated [Ca2+]i is reduced in parallel with IP production after chronic E. Furthermore, in contrast to synaptosomes and PC12 cells, N1E-115 neuro­blastoma do not exhibit an increased resting [Ca2+]i subse­quent to acute E exposure. (Supported by the Dept. of Vet. Affairs).
636.5 ETHANOL-INDUCED CHANGES IN 3H-GABA RELEASE FROM SUBSTANIA NIGRA AND SUPERFICIAL COROLLICUS. Joanna Pobell, Minzer K., Lawrence S. and Amy Shenker*. Dept. Pharmacody-
namics, Univ. Florida, Gainesville, FL.

An increase in neuronal transmission in the GABAergic striatal-

gional pathway and a decrease in GABAergic transmission in the

igroccellular pathway both are correlated with decreases in seizure

sensitivity. If the relation is causal, then the anticonvulsant effects of

ethanol should have opposite actions on GABAergic transmission in

SN and SC and the reverse should occur during ethanol withdrawal.

We measured the effects of in vivo ethanol on pre- and postsynaptic

measures of GABA transmission in SN and SC from naive rats and in

rats given ethanol in their drinking water for 24 days and then with-
drawn for 24 hrs. While ethanol inhibited 3H-GABA release from slices of

SC at low concentrations (50-200 nM), 500 nM was required to

inhibit release from SN. In fact, release was increased by low concen-

trations of ethanol in SN. We next measured these parameters in SN

and SC from rats that had a mean ethanol consumption of 9.4 g/kg

body weight/day. Ethanol inhibition of release in SN from ethanol-

treated rats was greater than in the control rats whereas ethanol inhibi-

tion of release from SC was less in ethanol-drinking rats compared to

control rats. When 2-pTSBTP binding was measured using quantitative

autoradiography, there was a greater binding of binding sites in stria-

tum of ethanol-treated rats compared to controls. Thus when ethanol is

acting as an anticonvulant, it increases release in SN and decreases

release in SC, but when animals are sensitized to seizures by ethanol

treatment, release is decreased in the nigrostriatal pathway and in-

creased in the striatonigral pathway. (Supported by FHS AA 08026)

636.7 THE EFFECT OF ETHANOL AND NITROUS OXIDE ON CEREBELLAR GRANULE CELLS AND PURKINJE CELLS. C.-F. Hsiao, R. Huang* and C. Huang. School of Basic Life

Sciences, Univ. Missouri-Kansas City, Kansas City, MO 64110

Among the initial manifestations of ethanol intoxication are the
delay in reaction times, the impairment of fine motor skills, and
the deterioration of motor coordination and mental abilities. We
previously showed that ethanol or nitrous oxide severely inhibited
neuronal activities in the granule cell layer of the cerebellum in
the cat. To further identify the effect of ethanol and nitrous oxide
on specific cell types, we have combined the method of intracellular
recording/injection with ethanol delivery (0.3g/kg IV) or nitrous oxide anesthesia (66%). Ethanol inhibited the

spontaneous discharges and auditory responses of most, but not
all, granule cells. The spontaneous discharges of Purkinje cells
were either suppressed or enhanced by ethanol. Occasionally, the
amplitude of action potentials also seemed to be affected. On the
other hand, nitrous oxide abolished the spontaneous discharges
and auditory responses of all granule cells. Some Purkinje cells
were excited, others were inhibited, and still others showed no
effect. In conclusion, ethanol and nitrous oxide have significant
but different effects on the spontaneous discharges and responses
of most cerebellar granule cells and Purkinje cells. (Supported by
PHS grant AA07643)
636.11

ETHANOL ENHANCES GABA RECEPTOR-MEDIATED CHLORIDE CURRENTS IN CHICK CEREBRAL CORtical NEURONS. J.N. Remoldos and A. Falaschi. Faculty of Medicine, Memorial University of Newfoundland, St. John’s, Nfld., Canada A1B 3V6.

Primary cultures of cerebral cortical neurons were prepared from seven day old chick embryos. The effect of ethanol on GABA-activated membrane currents was examined using whole-cell voltage-clamp recording in cells maintained for 2-25 days in vitro. Cells were continuously perfused (1-2 ml/min) with oxygenated artificial cerebrospinal fluid (ACSF) containing (mM) 140 NaCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose, 0.5 mM tetrodotoxin, pH 7.3. Ethanol was diluted in extracellular solution (final concentration 0.5-50 mM) and applied by bath perfusion. Recording pipettes contained (in mM) 140 KCl, 2 MgCl2, 10 HEPES, 4 ATP, pH 7.3. GABA (50-500 μM) applied, by brief pressure pulses directly to the soma elicited a current which reversed at 0 mV and was blocked by 50 μM bicuculline. In 60% of neurons examined (n>100) ethanol caused a potentiation of the membrane current elicited by GABA. The threshold concentration of ethanol was 1 mM, and the effect of ethanol was maximal at 10 mM. In many cells, higher concentrations (40-50 mM) of ethanol inhibited GABA currents. These effects of ethanol were all reversible. Cells obtained from individual embryos had similar responses to ethanol, whereas cells obtained from different animals varied greatly in their sensitivity to ethanol. In cultures where ethanol enhanced GABA responses, the excessive buffering of intracellular calcium (by including 5-10 mM BAPTA in the recording pipette) attenuated the effect of ethanol.

Supported by the Medical Research Council of Canada.

636.12

THE EFFECTS OF ETHANOL ON CULTURED SEROTONERGIC NEURONS AND ASTROGLIA. R.K. LeDour and M.J. DruesNeuroscience Program, Loyola U. of Chicago, Stritch School of Medicine, Maywood, IL 60153.

The effects of ethanol exposure on the development of serotonergic neurons were assessed by assessing the influence of 50, 150 and 300 mg/dl of ethanol on cultured fetal rhombencephalic cells. Rhombencephalic cells were removed from rat embryos on embryonic day 14, and cultured for 2 days in the absence of ethanol and an additional 4 days in the presence of ethanol. Cultured rhombencephalic neuronal and astroglial cells were assessed for serotonin (5-HT) uptake, 5-HT content, number of 5-HT immunoreactive cells, and DNA and protein content.

The results of these experiments demonstrate that exposure of cultured rhombencephalic neuronal cells to an ethanol concentration of 50 to 300 mg/dl does not affect 5-HT or DNA content, or the proportion of 5-HT immunoreactive cells. In addition, DNA content is unchanged in ethanol-exposed astroglial cells. However, 300 mg/dl of ethanol significantly reduces protein content and 5-HT uptake per cell to approximately the same extent in both neuronal and astroglial cells. Thus, it appears that at these ethanol concentrations examined ethanol does not produce cell death. However, ethanol does inhibit protein synthesis in both neuronal and astroglial cells. This decreased protein synthesis is reflected in decreased 5-HT uptake by both cell types.

636.13

CALCIUM-DIACETYL HOMOTAURINE (Ca-AOTA) ALTERS ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL PYRAMIDAL NEURONS (HPNs) IN VITRO. S. F. Mendu and K. Zalewski. University of Guelph, Guelph, Ontario, Canada.

Ca-AOTA has shown promise in preventing relapse in abstinent alcoholics. However, its mechanism of action is unknown. As ethanol can alter synaptic transmission and several ionic conductances in hippocampal neurons, we examined the effects of Ca-AOTA on HPNs in a slice preparation. We used standard intracellular recording or whole-cell "patch-slice" recording methods, in current- or voltage-clamp mode. Ca-AOTA had small concentration-dependent effects on membrane potentials and afterhyperpolarization amplitudes, hypopolarized and had no effect in equal numbers of cells, whereas 100-500 μM Ca-AOTA slightly hyperpolarized most HPNs. Under voltage clamp (-60 to -47 mV holding potentials; 5-25 mV hypopolarizing steps), Ca-AOTA (10-100 μM) had little reproducible effect on membrane potentials (which are reduced by ethanol), but caused an outward current and a large conductance increase. In contrast, Ca-AOTA elicited a net inward current (with conductance increase) in whole-cell recordings with TEA and Cs+. 50-100 μM Ca-AOTA reduced (by 5 to 57%; n=6) the TTX, 50-300 pM Ca-AOTA (like Cd++ 100 μM) blocked Ca++-dependent action potentials and hyperpolarizations evoked by depolarizing current. In whole-cell voltage clamp with TEA and Cs+, 50-100 μM Ca-AOTA reduced (by 5 to 57%; n=6) the peak Ca++-sensitive inward currents evoked by 20-65 mV depolarizing steps from holding potentials of 40 mV. These results suggest that Ca-AOTA, like ethanol, has multiple actions on HPNs, including activation of an uncharacterized K+ conductance and inhibition of a Ca++ current. These mechanisms could account for the clinical efficacy of this agent. (Supported by USPHS AA11642 and LIPHA, France.)

636.15

KCI-EVOKED GABA RELEASE UNCHANGED IN IN VITRO HIPPOCAMPUS AFTER CHRONIC FLURAZEPAM TREATMENT. E.L. Tieso and X.H. Xie. Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43660.

Electrophysiological studies in CA1 region of in vitro hippocampal slices from benzodiazepine-pretreated rats showed markedly reduced spontaneous and evoked current components were reduced in a dose-dependent manner 

636.16

CALCIUM-DIACETYL HOMOTAURINE (Ca-AOTA) ALTERS ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL PYRAMIDAL NEURONS (HPNs) IN VITRO. S. F. Mendu and K. Zalewski. University of Guelph, Guelph, Ontario, Canada.

636.17

CALCIUM-DIACETYL HOMOTAURINE (Ca-AOTA) ALTERS ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL PYRAMIDAL NEURONS (HPNs) IN VITRO. S. F. Mendu and K. Zalewski. University of Guelph, Guelph, Ontario, Canada.

636.18

KCI-EVOKED GABA RELEASE UNCHANGED IN IN VITRO HIPPOCAMPUS AFTER CHRONIC FLURAZEPAM TREATMENT. E.L. Tieso and X.H. Xie. Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43660.

Electrophysiological studies in CA1 region of in vitro hippocampal slices from benzodiazepine-pretreated rats showed markedly reduced spontaneous and evoked current components were reduced in a dose-dependent manner.

636.19

KCI-EVOKED GABA RELEASE UNCHANGED IN IN VITRO HIPPOCAMPUS AFTER CHRONIC FLURAZEPAM TREATMENT. E.L. Tieso and X.H. Xie. Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43660.

Electrophysiological studies in CA1 region of in vitro hippocampal slices from benzodiazepine-pretreated rats showed markedly reduced spontaneous and evoked current components were reduced in a dose-dependent manner.
636.17

EFFECTS OF ETHANOL AND CHLORIDAZEPoxide ON INHIBITORY PROCESSES IN THE FASCIA DENTATA AND HIPPOCAMPAL AREA CA1 IN RAT BRAIN SLICES

W. R. Proctor and T. V. Damledt. Veterans Admin. Medical Research Services and University of Colorado Health Sciences Center, Denver, CO.

Previous studies have shown that ethanol (EtOH) has effects on the dopaminergic system of the rat striatum, however, few studies have explored the direct effects of locally-applied EtOH on dopamine (DA) nerve terminals. In the present study, we used high-speed chronocarposmotron recording techniques using Nari-coated carbon fiber electrodes coupled with pressure ejection of EtOH to investigate the effects of this drug on DA nerve terminals in the unitary and axon-anesthetized rat. Local application of EtOH (100 mM EtOH with 2.5 mM CaCl2, 25-200 mM) from a multibarrel microcapillary did not produce a detectable change in the extracellular levels of DA in the anterior striatum. However, potassium (70 mM)-evoked overflow of DA was seen to be reversibly inhibited (approx. 50%) by EtOH. In contrast, tyramine (20 mM)-induced DA overflow was not altered by EtOH in the same brain region. In addition, the clearance/diffusion of locally-applied DA was reversibly enhanced by EtOH. This effect was partially antagonized by locally-applied nomifensine. Taken together, these results suggest that EtOH exerts its effects on DA-containing nerve endings in the rat striatum by causing an increase in DA uptake. (Supported by USPHS grants NS09197, AG06434, AA09515 and AA0101.)

636.18

ETHANOL INCREASES GABA MODULATED CHLORIDE CURRENT IN CORTICAL NEURONS IN RAT BRAIN SLICES

W. R. Proctor and T. V. Damledt. Veterans Admin. Medical Research Services and University of Colorado Health Sciences Center, Denver, CO.

Previous studies have shown very little effect of acute ethanol application on intracellular responses in rat hippocampal slices. In particular, ethanol did not enhance the GABA receptor mediated chloride current recorded in CA1 pyramidal neurons; however, depolarizations elicited by local application of NMDA were significantly reduced by 80 mM ethanol superfusion. The present study was designed to examine the effects of ethanol on pyramidal neurons located in the rat hippocampus. Using conventional "sharp point" microelectrodes (60-80 MΩ), 80 mM ethanol superfusion in the present study, we used high-speed chronocarposmotron recording techniques using Nari-coated carbon fiber electrodes coupled with pressure ejection of EtOH to investigate the effects of this drug on DA nerve terminals in the unitary and axon-anesthetized rat. Local application of EtOH (100 mM EtOH with 2.5 mM CaCl2, 25-200 mM) from a multibarrel microcapillary did not produce a detectable change in the extracellular levels of DA in the anterior striatum. However, potassium (70 mM)-evoked overflow of DA was seen to be reversibly inhibited (approx. 50%) by EtOH. In contrast, tyramine (20 mM)-induced DA overflow was not altered by EtOH in the same brain region. In addition, the clearance/diffusion of locally-applied DA was reversibly enhanced by EtOH. This effect was partially antagonized by locally-applied nomifensine. Taken together, these results suggest that EtOH exerts its effects on DA-containing nerve endings in the rat striatum by causing an increase in DA uptake. (Supported by USPHS grants NS09197, AG06434, AA09515 and AA0101.)

636.19

PSYCHOTROPIC AGENTS: ANXIETY

637.1

ANXIOPHOBIC AND CARDIOVASCULAR EFFECTS OF CCK-4 IN MONKEYS ARE BLOCKED BY THE CCK-B ANTAGONIST LY262691. R. M. Palmour*, G.A. Geierth*, Dept. of Pharmacology and Psychiatry, Univer. of Colorado Health Sciences Center, Denver, CO.

Supported by AA03527 and the V.A. Medical Research Services.

637.2


Supported by AA00101 and AA06522.
637.3
The R(+) isomer of zacopride has been shown to be more potent than the S(−) isomer in various in vivo (Barnes et al., 1990). In addition S(−)zacopride can attenuate the actions of R(+)zacopride (R. Naylor, personal communication). This study investigates the interaction of R(+) and S(−)zacopride emphasising the duration of the pretreatment time.
R(+)zacopride (1 μg/kg) reduced avertive responding (time in the dark reduced 61% to 24%, P<0.01). S(−)zacopride (10 μg/kg) given as a 0.5 or 10 min pretreatment failed to modify this response (time in dark 35%, 39% and 42% respectively). However, when S(−)zacopride was given as a 30, 40 or 60 min pretreatment the reduction in avertive behaviour was abolished (time in dark 63%, 63% and 65% respectively).
This study confirms the ability of S(−)zacopride to antagonise the anxiolytic action of R(+)zacopride and emphasises the importance of selecting a suitable pretreatment time for interaction studies.
Barrett JM et al. Pharmac Biochem Behavior (1990) 37,717-727

637.5
Novel compounds acting as partial agonists at benzodiazepine (BZ) receptors show only some of the effects typical of full agonists, making them candidates for anxiolytics without accompanying sedation, muscle relaxation, or dependence liability. Several partial agonists were examined for anxiolytic activity and degree of similarity to the full agonist midazolam. The BZ derivatives Ro 16-6028 (bretazenil) and Ro 17-1812, and the α2 antagonist WY-50,324 (10.0 mg/kg i.p.) produced a hypothamic response indicative of 5-HT1A agonism, but the magnitude of this response decreased over the course of repeated administration with both compounds.
In the aversive food presentation test, EMD 56551 was anxiolytic as did bupropine (10.0 mg/kg i.p.). At the same doses, WY-50,324 and bupropine also produced a hypothamic response indicative of 5-HT1A agonism, but the magnitude of this response decreased over the course of repeated administration with both compounds. The S(-)- and R(+)-anxiolytic activity of WY-50,324 was still evident after chronic administration. As expected, the S(-)-anxiolytic antagonist, etanserin, did not demonstrate 5-HT1A agonist or antagonistic activity after repeated administration. Changes in receptor sensitivity, as measured by selective agonist challenges at S(-)- and R(+)-receptors, did occur after chronic administration.
WY-50,324 (10.0 mg/kg i.p.) in the rat. Chronic pretreatment failed to modify this response (time in the dark reduced 61% to 24%, P<0.01). S(-)zacopride (10 μg/kg) reduced avertive responding reached approximately 10.0 mg/kg). By comparison, chlordiazepoxide (1.0-10.0 mg/kg) was more potent than the S(-) to reduce aversive behaviour (time in the dark reduced 61% to 24%, P<0.01).

637.7
ANXIOLYTIC ACTIVITY OF FLESINOXAN IN ANIMAL MODELS. B. Oliver, A.M van der Poel1, J. Mez, J.A.M. van der Heiden and J. Schiper. Dept. Pharmacology, Daphne, P.O. Box 900 1380 DA Weesp, The Netherlands.
Flesinoxan selectively binds to the 5-HT1A receptor (Ki= 1.7 nM). Flesinoxan has been studied in anxiolytic models in mice where exploration of the novelt environment is suppressed by punishment. In the 4-plate test, where ambulatory activity is punished by a mild foot-shock, flesinoxan and other 5-HT1A agonists elevate ambulatory activity.
Similar results are obtained in the light-dark model, where exploration of mice is inhibited by bright illumination. In another anxiety model in mice, which is based on stress-induced hyperthermia, flesinoxan is active at doses of 1 mg/kg i.p. and higher. Also 8-CMP-DPAT and buspirone are active in this model (0.3 mg/kg s.c. and 10 mg/kg p.o.), whereas ipsapirone does not show an effect even at 20 mg/kg.
Anxiolytic properties of drugs can also be detected by the so-called separation-induced ultrasonic vocalisations of infant rodents. Low doses of benzodiazepines reduce ultrasonic vocalisations in rat pups. 5-HT1A agonists like buspirone and ipsapirone also reduce these separation induced anxiety calls. Flesinoxan is highly active (0.3-3 mg/kg i.p.) and far more potent than buspirone or ipsapirone in this test, which is indicative of anxiolytic activity in man.
In conclusion, flesinoxan is a highly potent partial 5-HT1A agonist. Based on the animal pharmacology data there are strong indications that flesinoxan may have potential anxiolytic properties in man.

637.8
EMD 56551 (5-methoxy-3-(4-(4-(4-methoxyphenyl)-1-piperazinyl)butyl)indolin) exhibits the following binding profile in vitro (receptor and IC50, respectively): 5-HT1A: 4 nM; 5-HT2: 750 nM; D2: 420 nM; affinity to benzodiazepine receptors was ≤ 0.1 nM. EMD 56551 inhibited forskolin-stimulated adenylyl cyclase (IC50: 2 nM), but antagonized the inhibitory effect of 8-CMP-DPAT in this model at 3 and 10 μM. SHPTT-accumulation in rat n.rape was inhibited dose-dependently (threshold dose 0.01 mg/kg sc, respectively). At the elevated plus maze and social interaction tests in rats, EMD 56551 was anxiolytic with bell-shaped dose–response curves (3–100 and 0.3–100 μg/kg sc, respectively). Foot-shock induced vocalization in rats was decreased with an ED50 of 100 μg/kg sc. In drug discrimination studies, EMD 56551 generalized to both the β-blocker WY-50,324 and an adrenoceptor blocker with an ED50 of 0.6 mg/kg ip. Moreover, EMD 56551 inhibited two-way shuttle box avoidance and prevented haloperidol induced catalepsy.
The results characterize EMD 56551 as a selective partial 5-HT1A-agonist with a high potency and a broad dose range as regards anxiety activity.

637.4
WY-50,324, a novel adrenergic agonist derivative, has a high affinity for 5-HT1A and 5-HT2 receptors. In vivo and in vitro studies have demonstrated WY-50,324 to be a 5-HT1A partial agonist and a 5-HT2 antagonist. Behavioral characterization has indicated that WY-50,324 has an antidepressant profile. The primary purpose of the current study was to assess 5-HT1A agonist activity of WY-50,324 during the course of repeated administration by assessing hypoactivity and the serotonin syndrome. WY-50,324 is a 5-HT2 antagonist activity after chronic (14 day) administration was determined by assessing the serotonin syndrome and head shaking in mice. WY-50,324 in vivo sensitivity was ascertained after chronic (14 day) treatment by administering selective agonists for these receptor subtypes and measuring behavioral responses associated with their activation. Standard 5-HT1A and 5-HT2 compounds were examined concurrently. In the rat, WY-50,324 (100 mg/kg ip.) continued to produce a 5-HT1A partial agonist activity (10.0 mg/kg ip.) as well as 5-HT1A antagonism, as did buspirone (10.0 mg/kg ip.). At the same doses, WY-50,324 and buspirone also produced a hypothamic response indicative of 5-HT1A agonism, but the magnitude of this response decreased over the course of repeated administration with both compounds. The 5-HT1A and 5-HT2 antagonist activity of WY-50,324 was still evident after chronic administration. As expected, the 5-HT2 antagonist, etanserin, did not demonstrate 5-HT1A agonist or antagonistic activity after repeated administration. Changes in receptor sensitivity, as measured by selective agonist challenges at 5-HT1A and 5-HT2 receptors, did occur after chronic administration.
WY-50,324 (100 mg/kg ip.) or buspirone (10.0 mg/kg ip.) in the rat. Chronic treatment (14 day) produced a supersensitive 5-HT1A hypothermic response. These results demonstrate that after repeated administration the in vivo pharmacological profile of WY-50,324 is unaltered i.e. 5-HT1A partial agonist and 5-HT2 antagonist activity is still evident.
IN VIVO VOLTMETRIC NEUROCHEMICAL PROFILE FOR THE TRIAZOLOBENZODIAZEPINE, ADINAZOLAM, E.T. Pelhan and A. Broderick, Massachusetts General Hospital (Kum J-910), Convent Ave & W. 138th St., N.Y., N.Y. 10031, U.S.A.

Adinazolam is a benzodiazepine (BDZ) agonist, an anxiolytic compound without sedative activity (Amsterdam et al., Psychopharmacol. 88 (1986) 484). However, adinazolam does not affect either noradrenergic or serotonergic reuptake inhibition (Lab+ et al., Neurosci. Lett. 54 (1985) 27). A 14-treated adinazolam semmechanically, semivoltametry was used with stearate working electrodes to detect norepinephrine (NE) and serotonin (5-HT) in hippocampus (CA3) of cerebral hydrazide anesthetized, male, virout-free Sprague Dawley rats for the characterization of the NE and 5-HT electrochemical signals are published (Broderick, P.A., Neurosci. Lett. 95 (1988) 275). Additional studies showed that the α-adrenergic agonist, clonidine (30 μg/kg ip) significantly decreased the NE signal (36%, p<0.05), whereas the α-adrenergic antagonist, yohimbine (5 mg/kg ip) significantly increased the NE signal (24%, p<0.05). Moreover, the triazolobenzodiazepine, adinazolam (10 μg/kg ip) significantly decreased the NE signal by 36.4% (p<0.05) and significantly decreased the 5-HT signal by 39.0% (p<0.05) at the end of one hour of study. These data are consistent with known actions for the BDZ’s (Daglish, D.W., Eur. J. Pharmacol. 49 (1978) 133; Sanghera, M.K. and German, D.W., J. Neural Transm. 57 (1983) 267). Importantly, expected increased extracellular levels of NE and 5-HT, consistent with measurement of reuptake inhibitory mechanisms, do not occur. Thus, adinazolam regulates presynaptic NE and 5-HT release mechanisms. (Supported in part by an Upjohn grant).

637.11
A COMPARISON OF BENZODIAZEPINE (BZ) RECEPTOR AGONISTS IN RAT BEHAVIORAL MODELS OF ANXIETY AND SEDATION. J.V.Cassella and M.D.Craigo. Neurogen Corporation, Branford, CT 06405.

Drugs interacting with the BZ receptor reduce anxiety-related behaviors in animals and also produce a variety of side effects. Although some rat behavioral models have accurately predicted anxiolytic effects of these compounds, the measurement of anxiety-related behaviors can be confounded by concomitant sedation or motivational changes. This study compares the effects of the BZ receptor full agonist and antagonist, and partial agonists RO 16-6028 and CGS-9896 in three very different rat anxiety models (Elevated Plus Maze(EPM), Lick Suppression, and Fear Potentiated Startle) and an independent test of sedation (Locomotor activity). Dizepam (0.2-2.0 mg/kg,IV) and alprazolam (0.06-0.5 mg/kg,IV) dose-dependently reduced locomotor activity while RO 16-6028 and CGS-9896 depressed activity at some doses (0.125-1.0 mg/kg,IV and 0.5-2.0 mg/kg,IV, respectively). In similar dose ranges, all the compounds were efficacious in the EPM but sedative effects clearly interfered with the anxiety performance in this task. All compounds increased the animal’s willingness to take shocks in the lick task, with dizepam and RO 16-6028 clearly superior in efficacy. However, these two drugs also significantly increased drinking from a non-shocked spout. Performance in the Potentiated Startle paradigm also varied among the four test compounds. For the purposes of drug discovery, a number of different behaviors, each possessing a minimal and varying sensitivity to different potential side effects should be employed. The overall profile of test results might best predict clinical efficacy of new test compounds.

637.13

Diazepam diffuses through the placental barrier of humans and mice, and accumulates in the mesencephalon, where it produces a delay in the neuroblastic differentiation of fetal mice, and a styylog raphe chronicit ination and loss some fibers in the neurobasal nucleus. We investigated if these histological alterations of the fetal mesencephalon occur in adult mice. Single daily doses (2.7 mg/kg) of diazepam were administered to CD-1 strain female mice, between the 6th and 17th day of gestation. A control group received equivalent volumes of saline solution. Both groups were exposed to a novel situation but develop a pronounced hyperactivity in a novel situation but develop a pronounced hyperactivity of 150 min. per day. A polydipsic rat consumed 3.4 times greater volume of water compared to a food deprived control rat. To determine if selective serotonin re-uptake inhibitors could decrease this schedule-induced polydipsia, animals were treated with either clomipramine (CM), fluoxetine (FLU) or the noradrenergic re-uptake inhibitor, desipramine (DMI), as a negative control. Each of these agents were administered daily for at least 21 days at a dose of 5 mg/kg, ip, to polydipsic rats 60 min. prior to testing. Animals administered DMI showed no decrease in polydipsic behavior throughout the testing period. However, after chronic dosing, CM and FLU significantly reduced polydipsia by approximately 50%. Since obsessive-compulsive disorder (OCD) and polidipsic behavior both involve an exaggeration of normal behavior, the polydipsia model may be relevant for the prediction of compounds useful in the treatment of OCD. This idea is strengthened by the finding that CM and FLU effectively reduce both polydipsic behavior in rats and clinical symptoms of OCD in humans, while DMI does not.
CAMPARATIVE STUDIES OF CALCIUM CHANNEL BLOCKERS EFFECTS ON CENTRAL FUNCTIONS AND THEIR PSYCHOLOGICAL DISORDERS.


The modulation of Ca** in the cerebellar granule cells of rats and the behavioral alterations resulting from treatment of Ca** blockers were examined to evaluate the central effect of Ca** blockers on the improvement of psychological dysfunctions. In hyperpotassium-induced Ca** efflux, the fluorescence of Ca** induced by Fura-2am in the granule cells was potentiated about 1.6 times higher than the steady state level. Fifty percent inhibition of this potentiation was seen in 5x10^-6 M of flunarazine (Flu), 2x10^-5 M of nicardipine (Nic), and concentrations higher than 5x10^-6 M of diltiazem, verapamil and nifedipine showed about 20% inhibition. In the behavioral studies, Flu and Nic demonstrated pharmacological properties similar to diazepam, but not chlorpromazine. Also both Flu and Nic treatment markedly inhibited the hyperactivity of spontaneously hypertensive rats caused by mild audiogenic stress-induced fear, and also prolonged the duration of immobility in forced swimming of both male and female rats. These results suggest that the pharmacological properties of Ca** blockers in the central nervous system are completely different from those in the peripheral nervous system, and some Ca** blockers may be effective in the treatment of anxiety.

PERTUSIS TOXIN POTENTIATES SEIZURES INDUCED BY PILOCARPINE, KAINIC ACID AND N-METHYL-D-ASPARTATE.


Previous studies have shown that lithium potentiates the in vivo response to cholinomimetics in rats, resulting in seizures at otherwise nonconvulst doses, but did not affect seizure activity induced by some chemical convulsants including kainic acid and NMDA (Ormanoly et al., Exp. Neurol. 111:356, 1991). In vitro experiments have suggested that lithium interferes with neurotransmitter metabolism by both the GABAergic and NMDA systems. The present study tested the hypothesis that selective inhibition of G-proteins by in vivo treatment with pertussis toxin would increase the sensitivity of isolated brain neurons to GA BA mediated convulsant activity. Pertussis toxin (2, 3 days) mimicked lithium in potentiating the convulsant response of test neurons at both non-convulsant and convulsant doses. The effects of pertussis toxin were dose-dependent and the extent of potentiation was over 13-fold, which was comparable to the effect of lithium. The seizures were prevented by pretreatment with atropine, phenobarbital or diazepam. L-Phenylisopropyladenosine and MK-801 also demonstrated anti-convulsant activity, with MK-801 also protecting the rats against the rapid death associated with pertussis toxin/pilocarpine-induced seizures. Thus, seizures were cholinergically initiated and were controlled by the same drugs as were lithium/pilocarpine-induced seizures. The results illustrate that in several respects the response to cholinomimetics are modified in a similar manner by lithium and pertussis toxin. However, pertussis toxin lacks the specificity of lithium as it also potentiated the convulsant effects of kainic acid and NMDA.

PHOSPHATIDYLSINOLISTOL HYDROLYSIS IN BRAIN MEMBRANES.


The hydrolysis of phosphatidylinositol (PI) was measured in brain membranes using the method of Wallace and Claro (J. Pharm. Exp. Therap. 255:1296, 1990) in which the hydrolysis of exogenous [3H]PI is measured. [3H]PI hydrolysis was stimulated by GTPγS concentration-dependently with a maximal stimulation of about 3-fold obtained with 3 μM. NaF concentration-dependently stimulated [3H]PI hydrolysis to a greater extent than did GTPγS, with a maximal effect of about 5-fold with 20 mM NaF. Carbachol alone or with NaF did not stimulate [3H]PI hydrolysis, but with GTPγS carbachol stimulated activity above that obtained with GTPγS alone. Pilocarpine, which is only slightly active in slices, stimulated [3H]PI hydrolysis in the presence of GTPγS to a similar extent as did carbachol. Inclusion of excitatory amino acid agonists with GTPγS and carbachol did not inhibit [3H]PI hydrolysis, in contrast to results in slices, but with GTPγS some agonists effectively stimulated [3H]PI hydrolysis.

In membranes prepared from chronic lithium-treated rats (4 weeks) carbachol stimulated [3H]PI hydrolysis to a similar extent as in controls but activation of G-proteins induced lower stimulation than in controls. These results demonstrate that membrane preparations are useful to study the modulation of [3H]PI hydrolysis.

SEIZURE-INDUCED PROTEIN TYROSINE PHOSPHORYLATION IN RAT BRAIN REGIONS.

M. S. Baird, R. S. Jope and G. V. W. Johnson. Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama, Birmingham, AL 35294.

Phosphorylation of protein tyrosines is an important regulatory process for cell signalling and other cellular functions. Rat brain regions were examined for altered protein phosphorylations, using Western blot analysis and microwave irradiation to limit postmortem alterations, after administration of two convulsants, lithium plus picrotoxin or kainic acid. Most phosphorytose proteins were unaltered by these treatments, but there was a large, specific increase in the tyrosine phosphorylation of a 40 kD protein. This elevation was evident in all three regions that were examined, the cerebral cortex, hippocampus and striatum. This increase occurred abruptly with the onset of generalized status epilepticus and remained elevated for at least 90 min. The majority of the tyrosine phosphorylated 40 kD protein was found in the cytosolic fraction. These results demonstrate a large, specific effect of chemically induced seizures on a single phosphorytyrose protein in rat brain.

EPILEPSY: BASIC MECHANISMS V


The D1 dopamine receptor (D1) agonist, SKF83933, has been reported by several groups to potentiate pilocarpine seizures. We report that a variety of D1 agonists induce forelimb clonus in rats and mice independent of cholinergic co-treatment. The compounds tested include isochromes (A68930, A68979, A70500) and the benzazepine, SKF89558. Each produced clonus with an ED50 value of from 3 to about 300-fold higher than its ED50 for inducing rotation in rats with unilateral 6-OHDA lesions. The response showed anamnestic selectivity for the active enantiomer of A68930. SKF83933, A68979, and SKF89558. The response was anticonvulsant activity in the mouse model of the chronic convulsive treatment of clonus, clonazepam, ziprasidone and acetylsalicylic acid. It is also sensitive to D1-selective (SCH23390, A69024, SCH53156) but not D2-selective (haloperidol, YM09152-2) antagonists. Neither D2 selective (LY171555, bromocriptine) nor mixed D1/D2 (apomorphine) agonists produced clonus in the dose range tested. Furthermore, co-administration of LY171555 or bromocriptine provided apparent protection from clonus as evidenced by a rightward shift in the dose-response curve to A68930.
638.3

GAMMA-HYDROXYBUTYRIC ACID INDUCED SPIKE AND WAVE DISCHARGES IN RATS: ELECTROPHYSIOLOGICAL AND AUTOBIOGRAPHICAL EVALUATION. P. B. Angelides, B. A. L. van der Walt, G. W. J. van der Walt, M. J. van der Walt, U. M. van der Walt. Dept. of Neuropsychiatry, Children's Hospital of Los Angeles, Department of Neurology, Univ. of Southern California, Sch. of Med., Los Angeles, CA.

γ-hydroxybutyric acid (GHB), a naturally occurring compound, is synthesized from γ-aminobutyric acid (GABA) and induces bilateral synchronous spike and wave discharges (SWD) in rats which resemble generalized absence seizures. The GHB model of absence seizure has been well characterized in our laboratory (Snead, Epilepsia 29:361, 1988). The purpose of the present study was to investigate the pattern of SWD generation in different layers of the neocortex (FCtx), thalamus, and hippocampus. In this study, the animals were implanted with bipolar electrodes in two different layers of FCtx, dorsal hippocampus (CA1), ventromedial prefrontal (VPL) nuclei of the thalamus. The FCtx (layer one) bipolar recording was synchronous with the other cortical epidural (monopolar) electrode recordings while FCtx (layer two) did not seem to participate in the synchronous SWD. The thalamus recordings were found to be synchronously fired with the cortical epidural recording during GHB induced SWD but VL was found to be silent. Our biochemical findings using quantitative receptor autoradiography suggested that even though the highest density of GHB binding sites (labeled with 3H-GHB) were present in the hippocampus (CA3>CA1>CA2), the bipolar recordings of hippocampus (CA1 field) were silent during GHB induced SWD. Similarly, the bipolar recordings of VL were silent despite the fact that the GHB binding sites were more abundant in VL than in the VPL where SWD appeared synchronous with the cortical epidural recordings. Interestingly, the distribution pattern of 3H-GHB in the brains from animals sacrificed during GHB induced SWD, showed a significant increase in 3H-GHB binding in both the layers of FCtx (~120%). In the thalamus, the VPL binding rose to ~116% compared to GHB-naive VL, whereas the increment in VL was approximately 80%. These findings suggest that GHB induced seizures in thalamus and cortex are directly involved in the generation of SWD in the model of absence.

638.8


The concentrations of inositol 1,4,5 triphosphate (IP3), cyclic AMP, and cyclic GMP were measured in rat brain regions after acute or chronic lithium administration, as well as after the cholinergic agonist pilocarpine alone or in combination with lithium at a dose which induces seizures only in lithium pretreated rats. Neither acute nor chronic lithium treatment altered the hippocampal or cortical concentrations of IP3. Pilocarpine increased IP3 after 20 min, and this response was enhanced in both acute and chronic lithium-treated rats. At 60 min after pilocarpine, IP3 was elevated further, and with pilocarpine plus lithium the IP3 concentration remained elevated in the hippocampus but it decreased in the cortex. During seizures induced by pilocarpine cyclic AMP increased in the cortex with acute and chronic lithium and in the hippocampus with acute lithium treatment. Acute and chronic lithium treatments both caused some reduction in cyclic GMP. Seizures induced by pilocarpine resulted in large accumulations of cyclic GMP, but these increases were much less with chronic lithium administration. In summary, acute lithium treatment appeared only to reduce IP3 and cyclic AMP concentrations, whereas both basal and stimulated cyclic GMP production were reduced by lithium administration.

638.4

THE ELECTROPHYSIOLOGICAL BASIS OF EPILEPTIFORM MAGNETIC FIELDS IN NEOCORTEX: SPONTANEOUS ICTAL PHENOMENA. S. H. Old and D. S. Barth. Department of Psychology, University of Colorado, Boulder, CO 80309-0345 (U.S.A.)

In a previous report from our lab, electrical measurements of epileptiform cellular currents produced by physiologically evoked interictal penicillin spikes in rat somatosensory cortex were directly compared to the extracranial magnetic fields these currents generate. The present study uses the same methodology to extend these observations to spontaneous interictal and ictal phenomena in rat auditory cortex, and provides a more realistic empirical foundation for physical models with which to interpret noninvasive neuromagnetic recordings of human focal seizures. These data indicate that seizure foci under 1 x 1 cm² in cortical surface area are capable of producing magnetic fields that may be recorded at extracranial distances similar to those used in humans. Furthermore, physical models based on the dipole approximation appear to be appropriate for the interpretation of ictal field phenomena in neocortex.
638.9


EFFECTS OF INTRACEREBRAL MICRONIZED GLIaxon INJECtions ON CHOLINERGIC AND NEURONAL ACTIVITY. T. S. S. Moore, M. S. M. Brudzynski, and S. M. Brudzynski. Department of Neurology, University of Alberta, Edmonton, Alberta, Canada.

638.10


638.11

BRAINSTEM-EVOKED SYNCHRONOUS DISCHARGE IN THALAMIC NEURONS DURING SLEEP IN NORMAL AND PENICILLIN-TREATED CATS. M. K. S. House, R. J. Zimmerman, and D. M. Mcintyre. Department of Neurology, University of Colorado, Denver, Colorado, USA.

638.12

EFFECTS OF db-CAMP AND Na and K CONCENTRATION IN MEDIUM ON THE Na,K-ATPase ACTIVITY AND INTRACELLULAR Na AND K CONTENTS OF CULTURED ASTROCYTES. V. G. V. Garant and D. P. Boddy. Department of Neurology, University of Utah, Salt Lake City, Utah, USA.
**638.15**

LOSS OF GABA-MEDIATED INHIBITION IN LITHIUM-PILOCARPINE MODEL OF STATUS EPILEPTICUS. J. Kapur, E.W. Lothman* and R.J. DeLorenzo, Department of Neurology, Medical College of Virginia, Richmond, VA and *Department of Neurology, University of Virginia, Charlottesville, VA.

Convulsive status epilepticus (SE) is a neurological emergency. The pathophysiology of convulsion generation is complex. This study uses a combination of electrophysiological and biochemical methods to compare the state of GABA-mediated inhibition in rats injected with lithium and pilocarpine to cause SE, with that in naive and lithium-treated controls. Paired pulse inhibition technique was used to evaluate GABA-mediated inhibition in the CA1 region of the hippocampus of urethane-anaesthetized rats. There is a left shift of inhibition vs interpulse interval curve in test animals when compared with the curve from the two control groups. High affinity \( ^{3}H \) muscimol binding sites in rat forebrain synaptic plasma membrane (SPM) fraction are decreased generating a saturation curve. The SPM from control (naive or lithium-treated) groups showed a high affinity for \( ^{3}H \) muscimol and saturation of binding sites as reported in the literature. No high affinity binding site for \( ^{3}H \) muscimol or their saturation were detected in SPM derived from the test (SE) animals. These results demonstrate a loss of GABA-mediated inhibition in lithium-pilocarpine induced SE. Loss of GABA-mediated inhibition may result from post-translational modification of the GABA receptor and can play an important role in emergence of SE.

**638.16**

DIFFERENTIAL CHANGES IN EARLY AND LATE PHASE OF INHIBITION IN DENTATE GYRUS FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID. Moshe Khayat, E. Pagani* and N.W. Millham, Life Sciences Division, Scarborough Campus, U. Toronto, Scarborough, Ontario, Canada, M1C 1A4.

Following activation of dentate gyrus granule cells by perforant path stimulation, both an early and a late phase of inhibition of the population spike measure can be demonstrated with the paired pulse technique. These two inhibitory phases are decreased by use and such changes in inhibition are thought to contribute to seizure development. We have monitored both phases during the development of status epilepticus following systemic administration of kainic acid. Subjects were freely moving rats which had been chronically implanted with perforant path stimulation electrodes and dentate gyrus recording electrodes. Consistent with previous reports, prior to the occurrence of the initial afterdischarge activity we found a progressive decrease in the early phase of inhibition measured at an interpulse interval (ipi) of 25 msec. In contrast, there was an unexpected increase in the late phase of inhibition which measured at a 300 msec ipi. We also observed a progressive broadening of the field potential, which was similar to a previously reported late NMDA component and can provide an explanation for the increase in the late phase of inhibition.

**NEUROMUSCULAR DISEASES**

**638.17**

KYNURENATE AND ETHANOL WITHDRAWAL

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Ethanol withdrawal in dependent rats reduced the cortical content of kynurenine (KYN) from 41.52±8 pmol/g tissue to 24.03±1 pmol/g tissue. KYN is a tryptophan metabolite able to inhibit glutamate neurotransmission by interacting with glycine at the level of the NMDA receptor ion channel complex. In order to understand whether brain KYN decrease was associated with the symptoms of ethanol withdrawal we administered nicotinylalalnine (NAL) to dependent animals. We have previously shown that NAL administration (200 mg/kg i.p.) to rats significantly potentiates tryptophan (200 mg/kg i.p.) and nicotinylalalnine (NAL) on the percentage of ethanol withdrawal seizures. The administration of NAL (100-400 mg/kg i.p.) to ethanol dependent C57 black mice was able to prevent withdrawal seizures, tremors and changes in body temperatures, evaluated according to Ritzman and Tabakoff, J. Exper. Ther., 199, 158-170, 1976.

It appears therefore that by correcting the withdrawal induced decrease of brain KYN it is possible to prevent symptoms of ethanol withdrawal.

**639.1**


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Duchenne muscular dystrophy (DMD), an inherited, lethal disease of young males, has no known cure or treatment. Myoblast transfer therapy (MTT), intramuscular injections of normal myoblasts into the dystrophic muscles, has been developed as a possible treatment to alleviate the symptoms of DMD. In this double-blind, first clinical trial of MTT, 11 male volunteers with DMD, aged 5 to 11, received normal diseased muscles, has been developed as a possible treatment to alleviate young males, has no known cure or treatment. Myoblast transfer therapy (MTT), intramuscular injections of normal myoblasts into the dystrophic muscles. After MTT the test muscles demonstrated functional improvement, whereas the control muscles showed a loss in strength. Six measurements of twitch and MVC amplitudes were obtained before and at 3 and 6 months after MTT. Twitch tensions were evoked by supramaximal, indirect electrical stimulation. A study of eleven normal adult volunteers was conducted to quantified by comparing the 5-sec maximum voluntary contraction (MVC) tensions could be measured. The results were as follows: (1) the mean muscle strength of each of the three muscles of the patients was significantly (p < 0.001) reduced compared with healthy subjects; knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg, and (2) 1/2 and 3/4 RT means of the MD patients (vs healthy subjects) were: knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg, and (3) RT means of the MD patients (vs healthy subjects) were: knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg, and (4) RT means of the MD patients (vs healthy subjects) were: knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg, and (5) 1/2 and 3/4 RT means of the MD patients (vs healthy subjects) were: knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg, and (5) 1/2 and 3/4 RT means of the MD patients (vs healthy subjects) were: knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg.

After the 6-month therapeutic trial of amitriptyline (50 mg/day), the mean muscle strength of each of the three muscles of the patients was significantly (p < 0.001) reduced compared with healthy subjects. The results were as follows: (1) the mean muscle strength of each of the three muscles of the patients was significantly (p < 0.001) reduced compared with healthy subjects; knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg, and (2) 1/2 and 3/4 RT means of the MD patients (vs healthy subjects) were significantly different at p < 0.01. It is concluded that amitriptyline may provide therapeutic benefit to patients with myotonic dystrophy.

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Since advanced glycation endproducts have been suggested to mediate hyperglycemia-induced microvascular hyperpermeability, and because aminoguanidine (AG) prevents their generation, we examined whether AG could prevent or ameliorate the physiologic and biochemical indices of streptozocin (STZ) induced experimental diabetic neuropathy. Four groups of adult Sprague-Dawley rats were studied: Group I, STZ + 25 mg/kg/d AG; Group II, STZ + 50 mg/kg/d AG; Group III, STZ alone; Group IV, Controls. We monitored conduction serially in sciatic-tibial and caudal nerves, nerve blood flow, oxygen free radical activity (conjugated dienes and hydroperoxides), and the permeability coefficient-surface area product to 125I-albumin. STZ diabetes (Group III) caused a 57% reduction in nerve blood flow, abnormal nerve conduction and amplitudes, and a 60% increase in conjugated dienes. Nerve blood flow was normalized by 8 weeks with AG (Groups I, II) and conduction was significantly improved, in a dose-dependent manner, by 16 and 24 weeks in sciatic-tibial and caudal nerves, respectively. The permeability coefficient was not impaired, suggesting a normal blood nerve barrier function for albumin, and oxygen free radical indices were not ameliorated by AG. We suggest that AG reverses nerve ischemia and more gradually improves their electrophysiology by attenuating vascular permeability. AG may have potential in the treatment of diabetic neuropathy.

639.4 HIND LIMB PARALYSIS INDUCED BY INTRASPINAL CORD INJECTION OF COLCHICINE. IMPROVED RECOVERY BY CALCIUM BLOC KERS. M. Bercaldi, E. Zanotti, and G. T. Gentile. Dept. Pharmacology and Pharmacognosy, Modena University, 41100 Modena, Italy.

The intraspinal cord injection of small doses (5-10 mg/kg) of colchicine (C) at the lumbar level induces urine retention and hind limb paralysis in a dose related fashion. The onset of paraparesis occurs 1 day after the injection of 5 mg of C. By 2-3 days the hind limbs are completely flaccid. Therefore, the condition is used to improve beaking almost normal by 21-24 days. Hence this model was used as a tool to study the physical mechanisms of the paralysis due to spinal cord injury and to test new pharmacological treatments. The motor and sensory deficits in C treated rats were evaluated 1,3,6,8,10,13 and 15 days after C injection by actimetric system and neurological scoring. By day 3 after C injection the paralysis reaches its maximum score: we found by RIA a decrease of Sub P in the spinal cord (0.35 ± 0.02 nmol/g prot. of controls) without changes of Dyrophin. This finding seems to be in line with the increase of Sub P in spinal cord injury and with the notion that Sub P antagonists may produce paralysis after their intrathecal administration. By radioreceptor binding we found also a reduction of the NMDA-related receptors labeled by [3H]-TCP and of sigma receptors labeled by [3H]-SKF 10047 associated with an enhancement of calcium binding sites labeled by [3H]-PN 200-110 in the spinal cord of C treated rats (353 ± 16.1 nmol/g prot. in controls). Since both Sub P and glutamatergic system are involved in calcium homeostasis, we performed pharmacological challenge by chronically injecting the NMDA noncompetitive antagonist MK-801 or calcium channel antagonists (PK-10668 and PK-200-110). While MK-801 worsened the spontaneous recovery, the calcium channel blockers strongly facilitated the motor and neuronal recovery of the C-induced paralysis.


Current recordings were made from the surface of mdx myotubes and acutely dissociated intact fibers. Mechanosensitive channels in skeletal normal muscle are closed at rest and open when the membrane is stretched. However, a subset of the mechanosensitive channels observed in mdx myotubes are highly active at rest. The channels which show the highest resting activity in mdx myotubes are open at rest but close when stimulated with stretch. As an extension of our earlier results we also examined the activity of mechanosensitive channels in fibers acutely dissociated from the flexor digitorum brevis (FDB) muscle. In contrast to the two forms of mechanosensitive channel activity seen in mdx myotubes, a single class of mechanosensitive channels are observed in FDB fibers. However, surveying the surface of FDB fibers for channel activity, similarly showed that mechanosensitive channel activity is higher in mdx FDB fibers than in normal FDB fibers. A process was observed in ~15% of the patches containing channels in mdx myotubes and FDB fibers, in which stretch first activated channels then subsequently became inhibited with stretch. This may be interpreted as a switch in mechanosensitivity from being activated to inactivated with stretch. Thus, although FDB fibers do not show stretch-activated channel from the onset of recording, as is the case for myotubes, they do, however, show channels that are inactivated with stretch after a process of conversion. Therefore, it is possible that the percentage of channels activated in stretch and the resting activity may have potential in the treatment of diabetic neuropathy.


Membrane-mediated excessive intracellular calcium accumulation (EICA) plays a fundamental pathophysiologic role in hereditary muscular dystrophy in animals, as well as in Duchenne muscular dystrophy (MDM). Since influx of intracellular enzymes (ALD, CPK and LDH) with high plasma activities, decreased adenine nucleotides (ADN) with reduced cellular energy charge (GEC) are the inevitable biochemical evidence of the dystrophic tissue with EICA, these variants were measured in the ventricular myocardium (HT) and rectus femoris muscle (RF) of 1 and 12-month-old male DH, and CHF-148 normal hamsters (NH). Glucose-6-phosphate (GEP) level was also monitored to assess the efficacy of glycolytic pathways in DH. Plasma ALD, CPK and LDH were increased in both young and old DH, however, the plasma activity fell significantly with age. Correspondingly, these intracellular energy producing enzymes were significantly diminished both in HT and RF of older DH, but not in younger animals. GEP was reduced progressively in DH (p<0.05). In response to accelerated Ca2+-pumping in dystrophic tissue, ADN and GEC were significantly lower in the HT and RF of older DH, but not in younger hamsters showing considerably less EICA. Thus, ADN and GEC inversely correlated with EICA in DH. We conclude that membrane-mediated influx of intracellular enzymes and depletion of cellular ADN and GEC occur in DH. The glycolytic pathway in DH appears to be suppressed significantly. (Supported by NIH AR-38540)


Membrane-mediated chronic cellular degeneration plays a fundamental pathogenetic role in hereditary muscular dystrophy. We have shown EICA, influx of intracellular enzymes and aberrant sarcocellular dyifornin distribution in DH. We studied whether this genetic membrane insufficiency is associated with enhanced lipid peroxidation and altered antioxidant enzyme activities in muscle of 1 and 12-month-old male DH and sex matched normal hamsters (NH). Lipid peroxidation (LP), glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were measured in post 10,000 X g supernatant of rectus femoris muscle. Compared to NH, LP increased by 6 (p<0.01) and 43% to decreased GR activity of 43% (p<0.003) and 34% (p<0.001) in young and old DH, respectively. However, GR activity was higher in older DH (p<0.01), compared to younger. GSH level increased by 58% in older DH (p<0.01) and 26% to cellular adaptive mechanism and regeneration, but not in younger DH. GSH-Px activity was comparable in younger hamsters, but a 21% drop was noted in older DH (p<0.02). We conclude that chronic pathobiology in DH is inherently linked to cellular antioxidant enzyme-mediated lipid peroxidation of membranes. Increased LP in dystrophic muscle is accompanied by decreased GR and GSH-Px activities. E elevated GSH content in older DH appears to provide insufficient protection against inevitable peroxidative damage and progressive cellular degeneration. (Supported by NIH Grant AR-38540)

639.8 SKELETAL MUSCLE LACTATE DEHYDROGENASE IS DECREASED IN MURINE MOTOR NEURON DISEASE. B. N. Mandell and J. M. Rade*. Dept. Neurology, The University of New Mexico School of Medicine, Albuquerque, NM 87131.

Lactate dehydrogenase (LDH) is the glycolytic enzyme that converts pyruvate into lactate and generates NAD+ and acetyl-CoA from the skeletal muscle. Skeletal muscle LDH activity decreases after surgical denervation. Based on this fact we explored whether LDH might be decreased in a model of muscular dystrophy. The mdx Wobler mouse, a natural murine model of denervation due to degeneration of spinal cord motoneurons. LDH activity was determined by an enzymatic spectrophotometric method using homogenized biopsies muscle samples from matched pairs of Wobler and normal littermates at 3, 7, and 15 weeks after birth. In each age group, LDH activity was lower in the mdx Wobler. The lowest activity occurred in the oldest age group. Differences were statistically significant by paired t-test in the 7-week and 15-week age groups (p<0.05), with no difference in the 3-week age group. These results demonstrate a progressive alteration of muscle energy metabolism in a natural model of motor neuron disease. Supported by grant R29NS27698 from NINDS, NIH to RNM.
639.9  Differential staining of rat pudenal motoneurons with an antibody specific to somatic motoneurons. David G. Wells1, Artoe Y. Chiu2 and Cynthia J. Fomunon1. 1Dept. of Anat. and Neurobiol., Univ. of Vermont, Burlington, VT 05405 and 2Div. of Neurosci., Beckman Research Inst. of the City of Hope, Duarte, CA 91010. 

In amyotrophic lateral sclerosis (ALS), somatic motoneurons, but not preganglionic sympathetic neuron degeneration. In human ALS, the nucleus in the sacral cord that innervates the external sphincters of the anus and urethra (Onuf's nucleus), is spared (Mansen et al., J. Neurol. Neurosurg. Psychiatry 40:464, "77). This variation has led to the suggestion that these neurons are similar in some way to preganglionic neurons. 

Recently, an antibody (MO-1) has been developed that specifically recognizes cholinergic preganglionic neurons in the rat caudal and sacral cord. This antibody does not stain preganglionic neurons (Urakami & Chiu, J. Neurosci. 10:620, "90). We have applied this antibody to the rat lumbar spinal cord to investigate the distribution of preganglionic somatic motoneurons in the rat. 

All motoneurons that contribute to the sciatic nerve abnormally express this antigen; this is not the case in either DM or DL. Two populations of cells were observed in both nuclei. One group was similar to preganglionic neurons in that they expressed the antigen minimally, if at all. The second group of cells, interspersed with the first group, clearly expressed the antigen, but to a lesser extent than motoneurons that project via the sciatic nerve. Whether these two groups reflect differential staining between spindler and non-spindler motoneurons is currently being investigated by rneurotransmitter labelin in conjunction with MO-1 immunohistochemistry.


Sympathetic neurotransmitter antibodies to acetylcholinesterase (AChE) cause a unique complement-mediated destruction of preganglionic sympathetic nerve terminals (Brimijoin and Lennox, PNAS 87: 9630, 1990). To further assess neurological integrity in this disorder, rats given murine monoclonal AChE-antibodies or normal mouse IgG (1.5 mg, i.v.) were repeatedly examined over 4 mo with a neurobehavioral battery of tests for all four limbs. Antibody-treated rats developed permanent eye-injury (0.3-0.5 mm) within 4 h, reflecting loss of preganglionic sympathetic terminals. 

Persistent pupillary constriction (miosis) was noted, in accord with previous evidence of preserved parasympathetic function. Weight gain was also depressed. Essentially normal functions included neuromuscular performance (e.g., grip strength, landing foot-splay, gait), excitationality (e.g., arousal, reactivity), locomotor activity, and sensorimotor responsiveness to specific stimuli. Similar effects were noted in both nuclei: the dorsomedial (DM), which innervates the external anal sphincter and m. bulbocavernosus, and the dorsolateral (DL), which innervates the external urethral sphincter and the m. ischiocavernosus (Schneider, J. Comp. Neurol. 192:567, "80). These results confirm the autonomic focus of immunologic damage.

639.12  GLOBAL BLOCKADE OF CATECHOLAMINE-RELEASE AFTER PREGANGLIONIC SYMPATHETIC MY BY ACETYLCHOLINESTERASE ANTIBODIES. S. Brimijoin1 and G. M. Tyce2. Depts. of 1Pharmacology and 2Physiology, Mayo Clinic, Rochester, MN 55905. 

Previous results in rats treated with antibodies to acetylcholinesterase (AChE) demonstrated complement-mediated destruction of preganglionic terminals in sympathetic ganglia with survival of postganglionic neurons. To assess the resulting deficits of sympathoadrenal function, experiments were designed to measure stress-induced release of catecholamines. Rats (6 per group) were given murine monoclonal antibody to AChE, or normal mouse IgG (1.5 mg). Four days later a cannula was placed in the femoral artery. On the 5th day, a blood sample was taken from the awake, unrestrained animals. After that day the rats were subjected to 10 min of mild stress by forced immobilization in padded restrainers, a second blood sample was drawn, and the rats were killed by pentobarbital overdose. 

Catecholamine determinations by HPLC in all basal samples showed very low levels of norepinephrine and epinephrine (<0.2 ng/ml) and very low levels of the deaminated product, 3,4-dihydroxyphenylethylene glycol (DOPEG, 0.7-1.1 ng/ml). In controls, immobilization-stress increased plasma norepinephrine and epinephrine up to 25-fold (net increase 2.0 & 4.6 ng/ml respectively). The norepinephrine-response in antibody-treated rats was much smaller (15% of control) and the epinephrine-response was negligibly ablated (5% of control). Antibody-treatment also markedly reduced the stress-induced rise in DOPEG, although it did not lower basal levels. We conclude that preganglionic immunosympathetic leads to sustained blockade of neurotransmission throughout the sympathetic nervous system. (Supported by NS18170)
639.15

Several clinic and pathologic alterations have been described in skeletal muscle in patients suffering from cancer with no muscle metastases. Some of these changes have been considered of neural origin. In support of this point of view, we describe in this work the nerve and muscular ultrastructural pathology in a case of paraneoplastic phenomenon associated with a carcinoma of cervix. A needle biopsy was taken from quadriceps femoris muscle in a 65 year-old female who presented proximal muscular weakness and wasting, associated with a carcinoma of cervix. The patient was treated only with radiotherapy. The changes observed were: some muscle fibers showed different degrees of atrophy and others looked normal. Atrophied fibers presented in motor end-plates destruction of axon terminal with preservation of subsynaptic regions. Neural damage was also observed in myelinated fibers outside the end-plate. Vascular alterations and mononuclear cell infiltration formed by lymphocytes, macrophages and mast cells were also seen. Our findings suggest that muscular damage in this case probably has a dual etiopathogenic mechanism, vascular and neural. Supported by CDCH of UCV, Fundación Polar and The British Council Venezuela.

639.16
ABNORMAL ISOMETRIC MUSCLE FORCE CONTROL IN REFLEX SYMPATHETIC DYSTROPHY. C.J. Hunker* and M. Backonja. Dept. of Neurology, U. of Wisconsin, Madison, WI 53792.

The etiology and specific pathophysiology of movement disorders associated with reflex sympathetic dystrophy (RSD) are unknown; central nervous system mechanisms and psychogenic origins have been proposed. Freund and colleagues (1978, 1984) have shown that submaximal isometric contractions provide a means of relating muscle force control to involuntary motoneuronal activity. Hunker et al. (1991), have demonstrated that submaximal isometric contractions can distinguish pathological from somatoform tremors. Maximal and controlled voluntary contractions were examined in 10 RSD patients via isometric wrist and thumb/index finger tasks along with accelerometer recordings from affected and unaffected limbs. RSD patients presented muscle weakness and motor abnormalities of dyskinesia, dystonia, synkinesia and 6.0 Hz tremor localized to the affected limb. Since movement disorders of central origin result from abnormal motor unit activity, involuntary muscle force aberrations are salient during submaximal isometric contractions; in contrast, psychogenic movement disorders, which are not due to involuntary motor unit activity, are absent under isometric conditions. These data support putative CNS pathophysiological substrates for the movement disorders associated with RSD.